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JOURNAL
of
ECONOMIC ENTOMOLOGY

Formerly Published by the
AMERICAN ASSOCIATION OF ECONOMIC ENTOMOLOGISTS

Now Published by the
ENTOMOLOGICAL SOCIETY OF AMERICA
A new organization formed by the consolidation of the two major entomological organizations in the U.S.A. and continuing publication of the Journal as Volume 46, 1953.

MENASHA, WISCONSIN

APRIL • 1953

Volume 46 Number 2

JOURNAL OF ECONOMIC ENTOMOLOGY

Published by

ENTOMOLOGICAL SOCIETY OF AMERICA

Formerly Published by American Association of Economic Entomologists

AT MENASHA, WISCONSIN

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A bimonthly journal published on the 15th of February, April, June, August, October and December, devoted to the interests of economic entomology and publishing the official notices and proceedings of the Entomological Society of America.

TERMS OF SUBSCRIPTION. To members of the Entomological Society of America in continental U.S.A. and its possessions $4.00. Members outside of the U.S.A.—$4.50. Non-members in the U.S.A., Cuba, Mexico and Canada—$10.00. Non-members in other countries—$10.00 plus 60¢ postage. Single copies $1.75 plus postage of 10¢ per copy.

BUSINESS COMMUNICATIONS of the Society and SUBSCRIPTIONS AND ADVERTISEMENTS to the Journal should be sent to the business manager, Ernest N. Cory, University of Maryland, College Park, Maryland.

MANUSCRIPTS and other copy for the Journal as well as all correspondence concerning Journal content, should be addressed to the editor *pro tempore*, L. M. Peairs, at West Virginia University, Morgantown, W.Va.

CHANGE OF ADDRESS must reach the office of the Business Manager 45 days prior to the month of issue to be effective for that month.

Entered as second class matter at the Post Office at Menasha, Wisconsin. Acceptance for mailing at special rate of postage under the provisions of Sec. 34.40, Par. (D), provided for in the Act of February 28, 1925, embodied in paragraph 4, Section 538, P. L. and R., authorized May 1, 1936

Printed in U.S.A.

ISSUED MAY 28, 1953

JOURNAL OF ECONOMIC ENTOMOLOGY
ENTOMOLOGICAL SOCIETY OF AMERICA
Formerly Official Organ American Association of Economic Entomologists

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Chemical Control of the Corn Rootworm[1]

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The corn rootworm, *Diabrotica longicornis* (Say), (sometimes called northern corn rootworm) is an important economic pest in the Middle West. Metcalf & Flint (1951) state that "this is perhaps the most important corn insect in the upper Mississippi Valley." Corn rootworms affect corn yield directly by damaging the plants so as to reduce ear size, and indirectly by causing plant lodging and subsequent ear losses when mechanically picked.

The only known method of corn rootworm control prior to 1948 was crop rotation. This often failed if the field was out of corn only one year (Webster 1913; Bigger 1932). Chittenden (1905), in writing about both southern and northern corn rootworms, stated: "For the rootworm the use of insecticides on growing corn is impracticable. In fact, we cannot reach the root-worm stage to any extent with poisons." However, Fulton (1946) reported that soil applications of DDT and benzene hexachloride gave promise of a practical means of protecting young corn plants from southern corn rootworm. Hill *et al.* (1948) found that soil applications of benzene hexachloride greatly reduced western corn rootworm, *Diabrotica virgifera* Lee, and northern corn rootworm populations, and almost eliminated root injury and lodging. DDT applied in the same manner was not effective.

Several questions remain about corn rootworm control, although we know that chemical control is possible. This study was an attempt to add to our knowledge about (1) the effective insecticides, (2) various methods of application, (3) the most practical rate for each method of application, (4) the most effective place of application, *i.e.*, surface, subsurface, near seed, etc., and (5) the time of application in relation to the other four factors.

Materials and Methods.—All of the insecticides tested were of the chlorinated hydrocarbon type. Formulations were selected on the basis of the methods of application used in the different experiments. The following were used in wettable powder formulations: 20 per cent aldrin, 25 per cent aldrin, 40 per cent chlordane, and 25 per cent dieldrin. Emulsifiable concentrates were used in the following formulations: aldrin, two pounds per gallon; BHC, one pound of gamma isomer per gallon; chlordane, eight pounds per gallon; dieldrin, one and one-half pounds per gallon; DDT, two pounds per gallon; and heptachlor, two pounds per gallon.

Broadcast applications.—Two fields were used for broadcast applications in 1952. The insecticides were sprayed on the plowed ground and disked in before planting. Emulsifiable concentrate formulations were used in both experiments.

Flurer field: This field in Wright County had a cropping history of three years of continuous corn on the south half, and corn, soybeans and corn on the north half. The insecticides were applied with a tractor-drawn power sprayer on May 3, 1952. The rate of application was three-fourths pound of technical material per acre. Aldrin, gamma BHC, chlordane, and dieldrin were included. Each plot, including the untreated controls, amounted to 0.61-acre, and was replicated four times. The fourth replication feel in the south half of the field but it was observed later in the season that the rootworm in-

[1] This investigation was supported in part by research grants from the Shell Chemical Corporation, New York, N. Y., and the Julius Hyman Company, Denver, Colorado (now a Division of the Shell Chemical Corporation) in both 1951 and 1952, and from the Velsicol Corporation, Chicago, Illinois, in 1952.
Journal Paper No. J-2214 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 1096.

festation in the north half was no lighter than that in the south half.

Jelske Cramer field: The cropping history of this Wright County field was mixed pasture in 1949, corn in 1950, and oats in 1951. Severe rootworm damage was evident in it in 1950. The insecticides were applied with a tractor-mounted power sprayer on May 6, 1952. Aldrin and gamma BHC were each used at one-half pound and one pound of technical material per acre, respectively. Each plot, including the untreated controls, amounted to 0.96 acre and was replicated three times.

Banding insecticides along the rows.— This was accomplished by four different methods. (1) The insecticides were mixed in sand and fertilizer, respectively, and applied with a starter fertilizer attachment at the time of planting. (2) The insecticides were sprayed over the planter shoe trench by means of a planter-mounted power sprayer manufactured by the Broyhill Company of Sioux City, Iowa. (3) The insecticides were sprayed in approximately six-inch bands along both sides of the rows on the soil surface after the corn had emerged. (4) The insecticides were mixed in commercial fertilizer and side-dressed along the rows after the corn had emerged.

Miller field: A heavy infestation of rootworms was observed in this Story County field in 1950, so it held promise of a good infestation for experimentation in 1951. Wettable powder formulations of aldrin (25 per cent), chlordane (40 per cent), and dieldrin (25 per cent), respectively, were mixed in 3-12-12 mineral fertilizer in such a manner that when the fertilizer application rate was 100 pounds per acre, the insecticide rate was one pound of technical material per acre.[2] This field was planted with a four-row planter with starter fertilizer attachments on May 23, 1951. A different mixture was put in each of the four distributor boxes, *i.e.*, three insecticide-fertilizer mixes and the regular fertilizer as a control. These attachments are designed in such a way that they cannot be readily shifted in position or emptied by dumping. The whole experiment included about ten acres of De Kalb Hybrid 666 and about six acres of De Kalb Hybrid 668.

Burroughs field: This Butler County field had been in continuous corn for at least five years, with a severe rootworm infestation reported in 1951. Wettable powder formulations of aldrin (25 per cent) and chlordane (40 per cent), respectively, were mixed in washed river sand in such a manner that when the application rate of insecticide-sand mixture was 100 pounds per acre, the insecticide rate was one pound of technical material per acre. The field was planted on May 17, 1952, using a four-row planter with starter fertilizer attachments. The aldrin mixture was put in one end-box and the chlordane mixture in the other. The two middle boxes were left empty as untreated controls. Therefore there were alternating pairs of treated and untreated rows. The mixtures were not interchanged in the fertilizer boxes, due to the difficulty involved in this procedure. A total of about 0.75 acre was treated with each insecticide.

Yorgensen field: This Boone County field had been in soybeans after corn in 1950 but severe rootworm damage occurred when it was planted to corn in 1951. The insecticides were applied with the planter-mounted Broyhill sprayer on May 8, 1952. Emulsifiable concentrate formulations of aldrin, chlordane, dieldrin, and heptachlor, respectively, were used. Each compound was applied at the rate of one-half pound of technical material per acre. Each treated plot, including untreated controls, amounted to 0.81 acre and was replicated four times.

College Farm field: This field in Page County had shown rootworm damage in 1949 and 1950, after which a portion of it was replanted to corn in 1951 for this experiment. The toxicants were applied with a knapsack sprayer on July 16, 1951 when the corn was about 36 inches extended height. Emulsifiable concentrate formulations of aldrin, gamma-BHC and DDT were used. Aldrin and gamma-BHC were each applied at one-half pound and one pound of technical material per care, respectively. DDT was used at three pounds of technical material per acre. The proper amount of insecticide was mixed in 1.5 gallons of water for each plot (75-foot row), and sprayed on the soil along the row in a band about six inches wide. Each treatment, as well as the untreated control, was replicated five times.

[2] Aldrin-fertilizer mixtures of this type have recently been registered by the Production and Marketing Administration for use in rootworm control.

John Cramer field: This Wright County field was in oats in 1951 following heavy rootworm damage in 1950. Here the insecticides were mixed in 4-16-8 mineral fertilizer in such proportions that when the fertilizer application rate was 160 pounds per acre the insecticide was applied at 0.5 pound of technical material per acre. The toxicants were wettable powder formulations of aldrin (20 per cent), chlordane (40 per cent), and dieldrin (25 per cent). Twenty-six days after the corn was planted on May 2, 1952, the field was side-dressed with the insecticide-fertilizer mixes. A new tractor-drawn deep-placement fertilizer applicator was adjusted to distribute a band of the mix along both sides of the row and 9 inches from it. The depth of application was set at about 3 inches. Six rows made one plot, totalling 1.5 acres. Each plot was replicated four times.

Collection of data.—Three criteria were used to measure rootworm control in most of the experiments. These were: (1) counts of larvae, pupae and newly-emerged adults in and around the roots, (2) lodging records taken just prior to harvest, and (3) corn yield estimates as determined by hand-picked samples. In 1952 plant stand counts were taken 6 to 8 weeks after the corn was planted in order to detect any deleterious effects on germination or early growth. No harmful results due to treatments were observed following any of the replicated treatments.

In making the insect counts six hills were chosen at random in each plot and the tops of the plants were cut off about 6 or 8 inches above the ground. Each hill was then sampled by digging out the stalks, roots and surrounding soil within a radius of 6 inches from the plants to a depth of about 8 inches. The soil was carefully removed from the roots, sifted, and thoroughly searched for larvae, pupae and newly-emerged adults. The roots were dissected for feeding larvae. In 1952 the counts were started on July 9 and finished on July 28. Because of the delayed growing season the counts in 1951 were taken from August 7 through 10.

Since varying amounts of rootworm feeding causes plants to root lodge and "gooseneck" to different degrees, lodging records were taken just prior to harvest. Slightly different procedures were used in 1951 and 1952. In 1951 normal plants were considered to be those leaning from zero to 15 degrees from the vertical position, whereas in 1952 those plants in the range from zero to 30 degrees were classed as normal. In 1951 plants leaning from 15 degrees to 45 degrees from the vertical position were rated as "slightly" lodged, while those leaning at an angle greater than 45 degrees were classed as "severe." In 1952 "slight" lodging was recorded for those plants leaning between 30 and 60 degrees, while those leaning at angles greater than 60 degrees were rated in the "severe" class.

Both of the 1951 experiments employed one-row plots. The readings were made on 150 plants in each treatment in each variety in the Miller field. Lodging records were made on the total number of plants in each plot in the College Farm field in Page County. In the 1952 experiments a random starting point was selected in each plot in each field. Beginning at this point the total number of plants, the total number of slightly lodged plants, and the total number of severely lodged plants in 40 feet of row were recorded. Similar records were then taken for the next 40 feet in the adjacent row, so that a single sample consisted of 80 row-feet.

The same procedure for making yield estimates was used for all but two experiments. No yield estimates were taken from the College Farm field in 1951. In the Flurer field (pre-plant broadcast application) 27-hill samples were hand-harvested from each plot. Moisture determinations were obtained electrolytically on a Tag-Heppenstall moisture meter. The total ear weight per plot was then adjusted to 15.5 per cent moisture, making possible the bushels-per-acre yield estimates by the use of simple algebraic ratios.

In all other experiments the plots were sampled by a procedure suggested by Professor P. G. Homeyer of the Iowa State College Statistical Laboratory.[3] A pair of rows was chosen in each plot and random starting points selected. Seventy-five pairs of hills were then counted off from the starting point. All the plants that produced ears in these 2 by 75 hill

[3] The authors are indebted to Professor Homeyer for his suggestions on sampling procedures, and for supervising the analyses of the results on which the conclusions of this paper are based.

areas were counted. The ears from every fifth pair of hills were counted, harvested, weighed and sampled for moisture determinations. From the field weights corrected for moisture the bushels-per-acre yield estimates were obtained by appropriate calculations.

RESULTS AND DISCUSSION.—*Broadcast applications.—Flurer field:* Broadcast applications of the four insecticides listed in Table 1 before planting produced highly significant decreases in rootworm numbers. The averages per hill were 26.5 for the untreated plots, and 3.9 for the treated plots. The differences in rootworm numbers between the different insecticide treatments were not statistically significant.

With the exception of the dieldrin treatment the plants in all of the treated plots were 100 per cent free from lodging (Table 1). The dieldrin plots showed an average of only 0.3 per cent slight lodging. The lodging averages for the untreated control plots were 12.7 per cent slight and 2.1 per cent severe.

None of the treatments resulted in a significant increase in yield. The average yield in the untreated plots was 55.3 bushels per acre. Although yield estimates for aldrin and chlordane were 5.0 and 5.5 bushels per acre, respectively, greater than that of the control, these differences were not statistically significant. The yield from the gamma-BHC plots averaged 1.8 bushels per acre lower than the control.

Jelske Cramer field: Aldrin and gamma-BHC, both applied broadcast before planting, caused highly significant decreases in rootworm populations (Table 2). However, there was no significant difference between the two compounds or between the two levels at which they were used. The 0.5 pound rate of either compound was apparently just as effective as the 1.0 pound rate. There was a reduction in the mean number of rootworms per hill from 12.1 in the untreated plots to 1.2 in the treated plots.

Table 2 shows that there was no lodging recorded for any of the treated plots, and there was relatively little lodging in the untreated plots. Slight lodging in the latter averaged 7.2 per cent and severe lodging 1.4 per cent. Even this small amount was sufficient to enable one to locate the untreated plots easily by walking across the plots.

An average yield of 70.5 bushels per acre was produced in the treated plots, as compared with 64.8 bushels per acre in the untreated plots (Table 2). All of the treatments were followed by a yield increase of 3.3 bushels or more per acre, but the differences were not statistically significant.

Banding insecticides along the rows.—Miller field: The use of insecticide-fertilizer mixes banded along the rows with a starter fertilizer attachment at the time of planting caused a highly significant reduction in the number of rootworms per hill, according to the analysis of the data for De Kalb Hybrids 666 and 668 (Table 3). There was no significant difference among the insecticide treatments in either hybrid. However, when the data from the two hybrids were pooled, not only were the rootworm numbers significantly lower in the treatments than in the untreated controls, but there was a significant difference among treatments. Since the average number of rootworms found in the treated plots was lowest in the aldrin plots, aldrin apparently was more effective than the other insecticides in reducing the rootworm population.

There was no difference in the amount of slight lodging between the treated plots and the untreated plots in either hybrid, but the differences in the amount of severe lodging were highly significant for both (Table 3). Almost 21 per cent of the plants were severely lodged in the untreated plots of De Kalb Hybrid 668, whereas one per cent or less of the plants in the treated plots were severely lodged. In De Kalb Hybrid 666 twenty-nine per cent severe lodging was recorded for the control, compared with an average of only 1.4 per cent in the treated plots.

The yield estimates from De Kalb Hybrid 666 show that the treatments were followed by a highly significant increase in yield. The average yield for the untreated plots was 27.2 bushels per acre, as compared to an average of 36.2 bushels from the treated plots. There was no clear-cut indication of one insecticide being more effective in this respect than either of the others. It should be pointed out that the very low over-all yields were the result of the unusually poor growing conditions of 1951.

The results with De Kalb Hybrid 668

varied slightly from those with Hybrid 666. The yield from the treated plots was significantly better than the yield from the untreated plots. The mean yields were 28.9 bushels per acre for the untreated controls and 35.8 bushels per acre for the treatments. Also there was a significant difference among the mean yields of the different treatments. Since the mean yield of aldrin was lower than the yields of either chlordane or dieldrin the latter compounds apparently were more effective than aldrin in increasing the yield in this instance.

Burroughs field: The heaviest infestation of rootworms observed in either 1951 or 1952 was found in this field. The insecticide-sand mixes applied with a starter fertilizer attachment at the time of planting produced a highly significant decrease in corn rootworm populations (Table 4). The mean number of rootworms per hill was 111.6 in the untreated plots, as compared with 22.5 in the treated plots. Aldrin was significantly more effective in reducing the rootworm infestation than was chlordane. Since sand instead of fertilizer was used in this experiment none of the benefits can be attributed to fertilizer effects.

The data on slight lodging showed essentially the same results as the rootworm counts. There was a significant difference between the amount of lodging in the untreated plots and that in the treated plots (Table 4). The mean amount of slight lodging was 21.2 per cent in the untreated plots, 7.9 per cent in the chlordane plots, and 1.8 per cent in the aldrin plots. Severe lodging amounted to 14.8 per cent in the untreated control plots, as compared to 0.5 per cent in the chlordane plots and none in the aldrin plots.

For some reason the aldrin plots, after having been significantly better than chlordane in this test all season, gave a yield estimate 10.2 bushels per acre larger than the control plots but 11.6 bushels per acre lower than that from the chlordane treatment (Table 4). A mean yield of 70.4 bushels per acre was produced in the untreated plots, as compared with 80.6 bushels per acre in the aldrin plots and 92.2 bushels per acre in the chlordane plots.

Yorgensen field: Spraying the insecticides over the planter shoe trench with the planter-mounted Broyhill sprayer resulted in a highly significant decrease in rootworm numbers in the treated plots (Table 5). An average of 49.4 rootworms per hill was found in the untreated plots, whereas a mean of only 2.8 rootworms per hill was present in the treated plots. There was no good indication that one insecticide was more effective in reducing the rootworm population than any other.

Table 5 shows that there was no lodging in any of the treated plots. There was more severe lodging than slight lodging in the untreated plots. Slight lodging averaged 5.9 per cent, while 6.3 per cent, of the plots were severely lodged.

Inadvertently the farmer planted three corn hybrids in this experiment, so that a yield estimate could not be obtained from all four replications. Two of the replications were planted with the same hybrid and yield estimates were obtained from them. However, the results must not be accepted as conclusive on the basis of two replications. Actually the yield estimates shown for aldrin, chlordane, and dieldrin are somewhat below the untreated control yield, while the yield following the heptachlor treatment was considerably higher. Both the rootworm counts and the yield estimates indicate that heptachlor showed up very well in this experiment.

In this field one-half pound per acre of gamma-BHC was applied in an unreplicated plot for observation. This considerably reduced the stand and retarded the growth. Stand counts showed an average of 1.80 plants per hill, as compared with an average of 3.13 plants in adjacent untreated controls. This may have been an excessive dosage with this method of application. For the present the Iowa recommendations state that BHC should be used only in broadcast applications for corn rootworm control. Over-dosing should be avoided with all materials and methods of application.

College Farm field: Only root lodging records were taken from this experiment where insecticides were sprayed in bands along the rows after the corn had emerged. The applications of aldrin and gamma-BHC, respectively, caused highly significant reductions in both slight and severe lodging. There was no significant difference in the effectiveness of aldrin and gamma-BHC in this experiment. The higher (1.0 pound) levels of application of these two insecticides were no more

Table 1.—Effect of aldrin, gamma-BHC, chlordane and dieldrin, applied broadcast and disked in before planting, on rootworm populations, per cent and degree of lodging and yield of corn. Wright County, Iowa, 1952. Dosage shown as ounces per acre.

Treatment	No. Rootworms per Hill (July 18–19, 23–25)	Root Lodging		Yield Bu./A[1] (Sept. 27)
		Per Cent Slight (Sept. 1)	Per Cent Severe (Sept. 1)	
Aldrin (12 ounces)	6.5	0	0	60.3
γ-BHC (12 ounces)	0.8	0	0	53.3
Chlordane (12 ounces)	5.9	0	0	60.8
Dieldrin (12 ounces)	2.3	0.3	0	59.8
Untreated control	26.5**	12.7	2.1	55.3

[1] Corrected to 15.5 per cent moisture.

effective than the lower (0.5 pound) levels under the conditions of this experiment. DDT was significantly inferior to the other two compounds in the reduction of lodging. Actually DDT was included only as a standard by which to judge the other compounds, since previous work in Nebraska had shown it to be relatively ineffective against rootworms. The lodging means for the different treatments are given in Table 6.

John Cramer field: Side-dressing of corn with insecticide-fertilizer mixes by means of a deep-placement fertilizer distributor was ineffective in controlling corn rootworms in this experiment. All treatments failed to give significant reductions in the rootworm populations (Table 7). This table shows an average of 19.8 rootworms per hill in the untreated control plots, as compared with an average of 16.4 per hill in the treated plot samples.

Table 7 shows that the greatest amount of slight lodging occurred in the untreated plots. An average of 14.1 per cent of the plants in these plots were slightly lodged, as compared with 2.9 per cent in the

Table 2.—Effect of aldrin and gamma-BHC, applied broadcast and disked in before planting, on rootworm population, per cent and degree of lodging and yield of corn. Wright County, Iowa, 1952. Dosage shown as ounces per acre.

Treatment	No. Rootworms per Hill (July 21–22)	Root Lodging		Yield Bu./A[1] (Oct. 8)
		Per Cent Slight (Sept. 9)	Per Cent Severe (Sept. 9)	
Aldrin (8 ounces)	1.9	0	0	76.8
γ-BHC (8 ounces)	1.4	0	0	68.1
Aldrin (16 ounces)	0.9	0	0	68.8
γ-BHC (16 ounces)	0.6	0	0	68.6
Untreated control	12.1**	7.2	1.4	64.8

[1] Corrected to 15.5 per cent moisture.

Table 3.—Effect of aldrin, chlordane and dieldrin, mixed with fertilizer and applied with a starter fertilizer attachment at planting time, on rootworm population, per cent and degree of lodging and yield of corn. Story County, Iowa, 1951. Dosage shown as ounces per acre.

Treatment	No. Rootworms per Hill (Aug. 7–10)	Root Lodging		Yield Bu./A[1] (Nov. 8)
		Per Cent Slight (Nov. 1)	Per Cent Severe (Nov. 1)	
De Kalb Hybrid 666				
Aldrin (16 ounces)	1.7	15.9	0.9	35.8
Chlordane (16 ounces)	3.5	19.2	1.8	34.7
Dieldrin (16 ounces)	4.1	20.3	1.4	38.1
Fertilized control	20.5**	23.0	29.1**	27.2**
De Kalb Hybrid 668				
Aldrin (16 ounces)	1.3	17.7	0.7	30.4
Chlordane (16 ounces)	1.7	25.8	1.0	36.7
Dieldrin (16 ounces)	2.0	18.2	1.0	40.4
Fertilized control	15.3**	24.2	20.6**	28.9*

[1] Corrected to 15.5 per cent moisture.

dieldrin plots, 2.3 per cent in the aldrin plots, and 2.2 per cent in the chlordane plots. Severe lodging was varied, with none in the chlordane plots, 2.2 per cent and 2.0 per cent in the dieldrin and aldrin plots, respectively, and 0.9 per cent in the untreated control.

The yields were not increased by any of the insecticides. In fact all of the yield estimates for the treatments were lower than the yield from the untreated control plots. An average of 63.4 bushels per acre was produced in the untreated plots, while an average of only 61.0 bushels per acre was harvested from the treated plots.

It appears that the insecticide mixes were placed too far away from the plants, and possibly too deep for effective rootworm control. Depth of the insecticide, especially when below the area of greatest damage would be especially important if the toxic vapors tended to go down rather than diffuse out laterally in the soil.

SUMMARY AND CONCLUSIONS.—1. Aldrin, gamma-BHC, chlordane, dieldrin,

Table 4.—Effect of aldrin and chlordane, mixed in sand and applied with a starter fertilizer attachment at planting time, on rootworm population, per cent and degree of lodging and yield of corn. Butler County, Iowa, 1952. Dosage shown as ounces per acre.

Treatment	No. Rootworms per Hill (July 15–17, 19)	Root Lodging		Yield Bu./A[1] (Oct. 11)
		Per Cent Slight (Sept. 10)	Per Cent Severe (Sept. 10)	
Aldrin (16 ounces)	5.1	1.8	0	80.6
Chlordane (16 ounces)	40.0	7.9	0.5	92.2
Untreated control	111.6	21.2	14.8	70.4

[1] Corrected to 15.5 per cent moisture.

Table 5.—Effect of aldrin, chlordane, dieldrin, and heptachlor, respectively, applied over the planter shoe trench with a planter-mounted Broyhill sprayer, on rootworm population, per cent and degree of lodging and yield of corn. Boone County, Iowa, 1952. Dosage shown as ounces per acre.

Treatment	No. Rootworms per Hill (July 9–12)	Root Lodging Per Cent Slight (Sept. 9)	Root Lodging Per Cent Severe (Sept. 9)	Yield Bu./A[1] (Oct. 9)
Aldrin (8 ounces)	2.3	0	0	36.8
Chlordane (8 ounces)	4.2	0	0	39.9
Dieldrin (8 ounces)	3.1	0	0	38.2
Heptachlor (8 ounces)	1.4	0	0	46.9
Untreated control	49.4**	5.9	6.3	40.2

[1] Corrected to 15.5 per cent moisture. Average of two replications.

heptachlor and DDT were the insecticides used experimentally. Results are reported from seven different experiments.

2. The toxicants were applied in the following ways: (1) broadcast and disked into the plowed soil prior to planting; (2) mixed in fertilizer and applied with a starter fertilizer attachment at the time of planting; (3) mixed in sand and applied similar to starter fertilizer; (4) mixed in fertilizer and side-dressed along the rows with a deep-placement machine after the corn had emerged; (5) banded over the planter shoe trench with a planter-mounted Broyhill sprayer; and (6) surface-banded along the rows after the corn had emerged.

3. Methods of collecting data included (1) counts of larvae, pupae, and newly-emerged adults in the soil, (2) root-lodging records of plants before harvest, and (3) corn yield estimates obtained by hand-picking at the end of the season.

4. Corn rootworm populations were significantly reduced by (1) broadcast

Table 6.—Effect of aldrin, gamma-BHC, and DDT, respectively, sprayed in bands along the rows after corn had emerged, on per cent and degree of lodging. Page County, Iowa, 1952. Dosage shown as ounces per acre.

Treatment	Root Lodging Per Cent Slight (Sept. 17)	Root Lodging Per Cent Severe (Sept. 17)
Aldrin 8 ounces	20.0	4.3
γ-BHC 8 ounces	24.6	3.0
Aldrin 16 ounces	21.3	2.4
γ-BHC 16 ounces	20.4	2.5
DDT 48 ounces	35.4**	12.2**
Untreated control	38.5**	15.3**

Table 7.—Effect of aldrin, chlordane and dieldrin, respectively, mixed in fertilizer and side-dressed with a deep-placement machine after the corn had emerged, on rootworm populations, per cent and degree of lodging and yield of corn. Wright County, Iowa, 1952. Applied at 8 ounces per acre.

Treatment	No. Rootworms per Hill (July 24, 26, 28)	Root Lodging Per Cent Slight (Sept. 9)	Root Lodging Per Cent Severe (Sept. 9)	Yield Bu./A[1] (Sept. 27)
Aldrin	17.4	2.3	2.0	59.0
Chlordane	19.8	2.2	0	63.0
Dieldrin	12.1	2.9	2.2	61.0
Untreated control	19.8	14.1	0.9	63.4

[1] Corrected to 15.5 per cent moisture.

application of insecticides before planting, (2) mixing the toxicants in either sand or fertilizer and banding the mixture along the rows with a starter fertilizer attachment, and (3) spraying the insecticides over the planter shoe trench with a Broyhill sprayer. Insecticides mixed in fertilizer and side-dressed with a deep-placement machine after the corn had emerged failed to reduce rootworm populations in a single experiment.

5. All insecticides except DDT, applied by all methods of application except side-dressing by deep-placement, were followed by significant decreases in root lodging.

6. Significant yield increases were recorded only from the experiment that used insecticide-fertilizer mixes applied as starter fertilizer, and the experiment that used insecticide-sand mixes applied in the same manner.

7. Aldrin apparently was more effective than either chlordane or dieldrin in reducing the rootworm infestation in the Miller field when the results from both hybrids were pooled. Also aldrin was more effective than chlordane in the Burroughs field, on the basis of both the rootworm counts and the amount of root lodging. Chlordane and dieldrin were significantly better than aldrin on the basis of the yield estimates for De Kalb Hybrid 668 in the Miller field, and chlordane was significantly better than aldrin on the same basis in the Burroughs field.

8. Aldrin and gamma-BHC, each at 8, 12 and 16 ounces per acre, respectively, and chlordane and dieldrin, each at 12 ounces per acre, were effective in controlling corn rootworms when sprayed broadcast on plowed soil and disked in before planting in 1952.

9. One pound of aldrin, chlordane, or

dieldrin per acre gave very good rootworm control in 1951, when mixed in fertilizer and applied as a starter fertilizer mixture. Aldrin and chlordane at the same rate but mixed in sand instead of fertilizer gave good rootworm control in 1952.

10. Good control was obtained with aldrin, chlordane, dieldrin, and heptachlor, respectively, at the rate of 8 ounces per acre, when applied in a band over the planter trench with a planter-mounted sprayer.

11. Aldrin and gamma-BHC at 8 ounces per acre, respectively, reduced root lodging in corn when the treatments were applied in a band on the soil surface along the rows after the plants had grown to an extended height of about 36 inches.

12. Chemical control of the corn rootworm is possible and practical with a variety of insecticides and three different methods of application, on the basis of the results reported here.

13. More work is needed with lower insecticide dosages with the three successful methods of application. Further experiments are also desirable with the two post-planting methods that have shown up relatively poorly in the tests to date.

14. Over-dosing should be avoided regardless of the choice of material and the method of application. However, the work to date indicates that most of the effective materials have a good margin of safety on corn.

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Control of Mites on Strawberries in Virginia[1]

R. N. HOFMASTER and D. E. GREENWOOD[2]

The control of mites is one of the most serious problems encountered by strawberry growers in southeastern Virginia. Past observations (1947–52) have indicated that the two-spotted mite, *Tetranychus bimaculatus* Harvey, and strawberry spider mite, *T. atlanticus* McGregor, are the species of major economic importance. These mites suck the sap from the strawberry plants and cause a general loss in vigor, yield reduction, and permanent stunting or death.

Insecticidal control has been complicated by the fact that damaging mite infestations quite often occur in winter or early spring. For the most part low soil surface temperatures, cold nights and variable weather conditions make it extremely difficult to obtain adequate early season protection. Dusting with sulfur, 1 per cent parathion in sulfur or 1 per cent tetraethyl pyrophosphate has given good control in late spring but 3 to 4 applications are usually necessary. In addition to the number of treatments, all of the insecticides commonly used in this area have one or more of the following limitations: ineffectiveness and foliage injury under certain weather conditions; difficulty in timing applications properly; a high mammalian toxicity; and the time and cost involved in treating.

Danielson (1949) reported an apparent simultaneous control of the two-spotted mite and weeds in strawberries with an herbicide, dinitro ortho secondary amyl phenol. Danielson & Hofmaster (1951) obtained excellent mite and weed control in cold weather on a commercial scale with a single application of the herbicide. Unfortunately, grower acceptance has been retarded due to the fact that expanded foliage is destroyed by this chemical, although the growing point is not injured.

Hofmaster & Greenwood (1951) summarized the results of 4 years' tests to control the two-spotted mite on straw-

[1] Contribution from the Entomology Department, Virginia Truck Experiment Station, Norfolk. Paper No. 117, Journal Series. Approved for publication, December 1, 1952.

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Table 1.—Results of spray tests with Systox for control of *Tetranychus bimaculatus*. Norfolk, Virginia, 1951.

A. TREATED MARCH 23. TEMPERATURE AT APPLICATION 55° F. PLANTS IN SEMI-DORMANT CONDITION

Systox[1] per Acre	Mites per 25 (0.3 Sq. In.) Leaf Samples — Days After Treatment: 2 — Number	18 — Number	45 (May 8) — Number	45 (May 8) — Per Cent Reduction	52 — Number	52 — Per Cent Reduction	Residue p.p.m.[2] May 8
0.5 pint	55.3	31.5	179.3	50.0	209.0	14.4	
1 pint	28.3	20.0	81.0	77.4	63.8	73.9	0.4
1 quart	10.0	9.0	40.0	88.8	37.5	84.7	0.1
2 quarts	9.5	2.0	9.0	97.5	16.8	93.1	—
Check	290.5	180.8	358.8	—	244.3	—	0.0
L.S.D. 5%	39.4	28.7	49.9		46.9		
L.S.D. 1%	55.3	40.3	72.7		68.4		

B. TREATED APRIL 4. TEMPERATURE AT APPLICATION 53° F. PLANTS SHOWING NEW FOLIAGE

Systox[1] per Acre	Mites per 25 (0.3 Sq. In.) Leaf Samples — Days After Treatment: 2 — Number	18 — Number	33 — Number	40 — Number	40 — Per Cent Reduction	Residue ppm[2] May 8
0.5 pint	27.8	18.0	181.0	243.3	−6.6	0.1
1 pint[3]	86.8	14.5	89.8	97.3	57.4	0.1
1 pint	12.8	7.8	64.3	31.0	86.4	0.1
1 quart[3]	9.8	5.0	49.8	45.8	80.0	0.1
1 quart	4.5	3.8	33.8	21.5	90.6	0.1
2 quarts	6.0	0.8	9.5	11.0	95.2	0.4
Check	322.3	164.3	358.3	228.3	—	0.0
L.S.D. 5%	66.7	49.0	38.8	48.3		
L.S.D. .1%	93.5	68.7	58.4	64.8		

C. TREATED APRIL 30. TEMPERATURE AT APPLICATION 70° F. STRAWBERRIES COMMENCING TO TURN PINK AND WERE HARVESTED MAY 8. PLANTS IN POOR GROWING CONDITION

Systox[1] per Acre	Mites per 25 (0.3 Sq. In.) Leaf Samples — Days After Treatment: 2 — Number	5 — Number	5 — Per Cent Reduction	15 — Number	15 — Per Cent Reduction	Residue ppm May 8[2]
0.25 pint	150.8	106.5	81.4	106.8	52.9	0.1
0.5 pint	79.3	68.8	88.0	50.0	77.9	0.1
1 pint[3]	94.7	106.8	81.3	63.5	72.1	0.1
1 pint	40.5	36.3	93.6	22.0	90.3	0.1
Check	490.5	570.8	—	226.8	—	0.0
L.S.D. 5%	36.8	40.9		27.4		
L.S.D. 1%	51.9	57.1		38.2		

[1] Principally the diethoxythiophosphoric acid ester of 2-ethyl mercaptoethanol, 32.1 per cent emulsion supplied by the Chemagro Corporation.
[2] Residues determined by Geary Chemical Co. by means of bioassay.
[3] Low volume rate of 15 gallons of water per acre; other treatments applied in 100 gallons of water per acre.

berries. Thirty-four formulations were tested and promising results were obtained with Ovotran, parathion, TEPP, malathon, dinitro caprylphenylcrotonate and sulfur. Combinations of parathion with sulfur and Ovotran produced significantly better results than any of these materials alone.

METHODS.—Due to the low growing nature of the strawberry plant, good coverage with sprays is difficult to obtain. For this reason all of the acaracides except Systox were compared as dusts. These dusts, unless requiring a special diluent, were prepared by adding the concentrate to pyrophyllite and mixing in a ribbon blade dust mixer for fifteen minutes. Dusts were applied by a rotary hand duster or by a motor driven tractor-drawn duster having the nozzles adjusted with three per bed and discharging under a 25 foot cloth trailer. Sprays were applied by a single nozzle knapsack-type hand sprayer operating at approximately 40 lbs. pressure or by a power take off weed sprayer operating at 50 lb. pressure. The treatments were replicated four times in plots three or more beds wide; records were taken from the center bed or beds. All observations and treatments were made on the Blakemore variety of strawberries.

The immediate effectiveness of the several acaracides was determined by selecting 25 infested leaves from each treatment replication, extracting a 0.3 square inch leaf plug and counting all living mites. In counts made later than a week after treatment the same procedure was followed with the exception that no attempt was made to select infested leaves.

TESTS IN 1951.—Preliminary greenhouse observations during the fall of 1950 indicated the possibility of controlling the two-spotted mite with a new systemic insecticide, Systox.[3] Potted strawberry plants were infested and then treated with various concentrations of this material. Dosages as low as 0.06 pint (32.1 per cent emulsion) per 100 gallons of water gave a good initial kill when applied as a foliage spray. However, the mites showed a tendency to build up rather rapidly at this low rate.

Two-spotted mite populations during the winter of 1951 were low and it was not until late March that damaging infestations were noted. Since Systox is translocated within the plant system it was thought that the temperature and also the growing condition of the strawberry plants at the time of treatment might influence the degree of mite control obtained. Accordingly, application dates were selected as follows: March 23, strawberry plants semi-dormant; April 4, plants showing a few green leaves; and April 30, berries turning pink. Inasmuch as mite control in the field is a long term proposition it was decided to test relatively high concentrations of Systox in an effort to obtain protection through the three to four week picking period. Application was by means of a hand operated knapsack type sprayer at 15 gallon and 100 gallon per acre rates. Treatment replicates were three beds wide and 100 feet long. The concentrations tested and results obtained are summarized in table 1.

Observation of table 1A indicates that 0.5 pint of Systox per acre applied approximately six weeks before harvest gave significantly poorer control at harvest time (May 8) than higher rates. By mid-harvest mite populations in the 0.5 pint plots were almost equal to the check with severe plant injury apparent. In table 1B there was little difference in the degree of control obtained with various concentra-

Table 2.—Results of late season treatments for two-spotted spider mite control. Norfolk, Virginia. April 30, 1950.

Treatment[1]	Mites per 25 (0.3 Sq. In.) Leaf Samples			
	May 5		May 15	
	Number	Per Cent Reduction	Number	Per Cent Reduction
Sulphenone, 10%[2]	84.0	87.3	39.5	89.0
Sulfur	49.0	92.6	49.8	86.1
Malathon, 4%[3]	36.0	94.6	84.5	76.4
Dinitro caprylphenyl-crotonate, 5%[4]	58.8	91.1	53.5	85.1
Ovotran, 5%[5]	184.5	72.1	49.0	86.3
1% Parathion—5% Ovotran	13.5	97.9	11.5	96.8
1% Parathion—Sulfur	12.5	98.1	12.8	96.4
Parathion, 1%	25.5	96.1	86.0	76.0
Aramite, 3%[6]	159.0	76.0	137.0	61.8
Systox, 0.5 pint	45.0	93.2	70.5	80.4
Systox, 1.0 pint	15.5	97.7	23.0	93.6
Check	661.8	—	358.8	—
L.S.D. 5%	34.1		35.7	
L.S.D. 1%	46.2		48.4	

[1] Systox treatments made on April 15 in 100 gallons of water per acre. All material except Systox applied as dusts on April 30 at approximately 35 lbs. per acre.
[2] *p*-chlorophenyl phenyl sulfone; Stauffer Chemical Company.
[3] S-(1,2-dicarbethoxyethyl) 0,0-dimethyl dithiophosphate; American Cyanamid Company.
[4] Karathane; Rohm and Haas Company.
[5] *p*-chlorophenyl *p*-chlorobenzenesulfonate; Dow Chemical Company.
[6] 2-(*p*-tert-butylphenoxy) isopropyl 2′-chloroetoyl sulfite; Naugatuck Chemical Division, U. S. Rubber Company.

[3] Principally the diethoxy thiophosphoric acid ester of 2-ethyl mercaptoethanol.

tions of Systox after 18 days. However, after 33 days a dosage of 0.5 pint Systox per acre showed a mite decrease of only 49.5 per cent and a population 6.6 per cent higher than the check at mid-harvest, one week later. This is in contrast to reductions of 82.1 and 86.4 per cent, respectively, for the 1 pint rate at the same time intervals. In 1C, one-fourth pint of Systox per acre applied 8 days before harvest reduced the number of *Tetranychus bimaculatus* 81.4 per cent at 5 days but failed to provide good protection later. Two weeks after treatment 0.5 pint and 1 pint of Systox per acre gave 77.9 and 90.3 per cent reductions, respectively. In general, poorer results were obtained with low gallonage applications (Table 1B and C).

The effects of plant condition and temperature on the degree of control obtained with comparable concentrations of Systox are not clear cut. It would appear however, that the immediate effectiveness of Systox is somewhat greater when the plants are actively growing. Under the conditions of these tests, temperature did not appear to play too important a role. For example, two days after treatment, 0.5 pint and 1 pint of Systox per acre gave significantly better control when applied at a temperature of 53° F. (Table 1B) than when applied at a temperature of 70° F. (Table 1C).

The long term protection afforded by Systox is rather clearly indicated by the fact that 1 pint of this material per acre showed a per cent reduction of 86.4 at mid-harvest, 40 days after treatment. The increased control (5–10 per cent) resulting from higher concentrations of Systox would not appear to be of importance from a practical viewpoint (Table 1B).

It will be noted that the mite population was declining by mid-May. This decrease was in accord with past observations in which it was found that two spotted mites reach a peak on strawberries at or during harvest and then decline so rapidly that they practically disappear during the next two or three weeks. This low level of population remains relatively constant until fall when a gradual build up occurs.

Previous tests (Hofmaster & Greenwood 1951) have shown that good mite control may be obtained with dusts of sulfur and parathion-sulfur in late season. Results from a small scale greenhouse test indicated that additional applications of these insecticides at 4 to 5 days intervals would greatly increase their overall effectiveness. Plots 75 feet long by 3 beds wide were established April 15 to determine the effect of varying the type and number of insecticidal treatments. Two Systox sprays were applied on this date but a 0.26 inch rain prevented dusting. Weather conditions remained unfavorable until April 30 at which time the dust treatments were applied. The temperature at the time of application was 65° F. with a relative humidity of 66 per cent. The weather became somewhat warmer during the next two days reaching a high of 68° F. on May 1, and 87° F. on May 2. Due to the lateness of the season it was decided to make only a single treatment. The results of these tests are presented in table 2.

The main objective of this experiment was not attained because of the delay in treatment and the data are presented mainly to show the long term reduction in mite populations obtained with a single application of Systox under unfavorable weather conditions. Apparently the 0.26 inch rainfall, which occurred one hour after treatment, had little effect on the action of this compound. Observation of table 2 also indicates that parathion-sulfur and parathion-Ovotran were outstanding for their rapidity of action and lasting protection. Ovotran alone was slower in action but showed promising residual qualities. Combinations of parathion with sulfur and Ovotran produced significantly better results than any of these materials separately. The phosphates gave excellent initial control but limited protection later. Sulphenone and dinitro caprylphenylcrotonate showed considerable promise.

TESTS IN 1952.—As indicated previously, one of the problems faced by strawberry growers in the eastern Virginia area has been that of mite control during the winter. In 1951 applications of Systox at temperatures of 53° to 55° F. proved very effective against *Tetranychus bimaculatus*. Unfortunately, due to a low mite population that year, no treatments were made until late March at which time night temperatures and soil surface temperatures were considerably higher than those normally occurring in winter. Therefore, it was decided to test Systox under as varied

Table 3.—Effectiveness of winter treatments with Systox for strawberry spider mite control. Oceana, Virginia, 1952.

A. TREATED JANUARY 16. TEMPERATURE AT APPLICATION 56° F. PLANTS IN SEMI-DORMANT CONDITION

	Mites per 25 (0.3 Sq. In.) Leaf Samples						
	Days After Treatment						
	2		4	18	35	68[2]	
Systox[1] Per Acre	Number	Per Cent Reduction	Number	Number	Number	Number	Per Cent Reduction
0.5 pint	55.8	73.9	28.5	35.5	60.3	138.0	49.2
1 pint	19.0	91.1	12.8	20.0	26.0	70.5	74.1
1 quart	7.5	96.5	4.5	5.0	4.5	27.5	89.9
2 quarts	3.0	98.6	2.0	2.3	1.8	8.8	96.8
Check	214.0	—	203.5	175.0	131.0	271.8	—
L.S.D. 5%	10.8		13.5	16.0	19.4	38.6	
L.S.D. 1%	15.1		19.2	23.1	27.2	54.1	

B. TREATED JANUARY 30. TEMPERATURE AT APPLICATION 25 TO 28° F. PLANTS AS NEARLY DORMANT AS THEY EVER BECOME IN THIS AREA

	Mites per 25 (0.3 Sq. In.) Leaf Samples					
	Days After Treatment					
	2		4	18	55[2]	
Systox[1] Per Acre	Number	Per Cent Reduction	Number	Number	Number	Per Cent Reduction
0.5 pint	82.3	52.4	70.8	45.5	122.5	43.7
1 pint	32.0	81.5	30.0	21.5	67.0	69.2
1 quart	3.5	98.0	4.0	4.0	13.3	93.9
Check	173.0	—	160.0	113.5	217.5	—
L.S.D. 5%	13.5		14.9	13.3	27.6	
L.S.D. 1%	19.5		12.5	19.1	39.7	

C. TREATED MARCH 31. TEMPERATURE AT APPLICATION 60° F. PLANTS SHOWING NEW FOLIAGE

	Mites per 25 (0.3 Sq. In.) Leaf Samples				
	Days After Treatment				
	3		7	22[2]	
Systox[1] per Acre	Number	Per Cent Reduction	Number	Number	Per Cent Reduction
0.5 pint	50.5	82.1	30.3	110.5	60.3
1 pint	25.5	91.0	12.3	27.0	90.3
1 quart	6.8	97.6	3.0	12.3	95.6
2 quarts	1.5	99.5	1.8	7.3	97.4
Check	282.5	—	318.0	278.0	—
L.S.D. 5%	33.3	33.3	39.1	30.5	
L.S.D. 1%	47.8	47.8	56.2	43.9	

[1] 32.1 per cent emulsifiable concentrate supplied by the Chemagro Corporation.
[2] Counts discontinued due to grower dusting through the plots. Samples of berries from all treatments in A, B and C showed no Systox residues at harvest May 12; residues determined by Pittsburgh Coke and Chemical Company by cholinesterase method.

and severe climatic conditions as possible during 1952.

Early in January, 1952 a field of strawberries heavily infested with the strawberry spider mite was located near Oceana, Virginia. Mites of all stages were present and areas of the field were almost completely destroyed. On January 16 a series of tests was begun comparing the effectiveness of Systox against *T. at-*

Table 4.—Results of Field scale applications of Systox for strawberry spider-mite control. Norfolk, Virginia, April 1, 1952.

	Mites per 50 (0.3 Sq. In.) Leaf Samples							
	Days After Treatment							
	2	18	38 (May 8)		56			
Treatment	Number	Number	Number	Per Cent Reduction	Number	Per Cent Reduction	Yield (Qts) per 100 ft. Row	Residue[2] ppm May 12
1.5 pints Systox[1]	3.0	4.8	47.0	95.4	594.5	60.0	38.0	0.0
Check	875.3	540.0	1032.3	—	1492.0	—	10.5	0.0

[1] A 48.1 per cent emulsion supplied by the Pittsburgh Agricultural Company. Applied in 35 gallons of water per acre.
[2] Residues determined by Cholinesterase method by Pittsburgh Coke and Chemical Company.

lanticus under several extremes of temperature and plant condition. The different concentrations of Systox, conditions at treatment and results obtained are given in table 3.

Table 3 shows that Systox was generally effective in controlling mites over a wide range of temperature and plant conditions. However, the combination of low temperature (25° to 28° F.) and dormant plants (Table 3B) reduced the relative degree of mite control at dosages below 1 quart per acre. It is interesting to note that almost identical results were obtained with the 1 pint rate in applications made approximately 11 weeks apart (Table 1A and C). In general, 0.5 pint of Systox per acre definitely failed to afford the long term protection required from early season applications. In fact, treatment with this rate under relatively favorable conditions (Table 3C) gave a mite reduction of only 60.3 per cent 22 days later. One pint of Systox per acre gave somewhat better initial results but showed a pronounced mite increase after approximately two months (Table 3A and B). Concentrations above 1 pint per acre kept mite populations well in check.

Field Scale Tests with Systox.—Field scale applications of Systox were conducted at Norfolk, Virginia April 1, 1952. One and one-half pints of Systox (48.1 per cent emulsion) per 35 gallons of water per acre were applied by means of a power takeoff weed sprayer equipped with flat spray (32 M) nozzles operating at 50 lb. pressure per square inch. Approximately

Table 5.—Results of early spring treatments for strawberry spider mite control. Norfolk, Virginia April 14, 17, 1952.

		Mites per 25 (0.3 Sq. In.) Leaf Samples					Mite[2] Damage to Foliage			
		Days After April 17 Treatment							Yield (Qts.)	
Treatment	Amount[1] per Pounds or Gallons	4	8	21	32 (May 19)	39 (May 26)	May 19	May 26	per 15 Ft. Row	Residue[3] ppm
		Number	Number	Number	Number	Number	Rating	Rating		
Dusts:										
Aramite, 3%[4]	37	40.8	21.0	26.0	33.5	176.3	1.5	2.9	10.2	
Sulfur	45	48.8	29.0	36.5	55.8	289.0	2.0	3.5	—	
Ovotran, 5%	35	95.0	37.0	42.8	62.0	223.5	2.0	3.2	—	0.4
Ovotran, 10%	33	88.8	24.3	16.8	39.5	167.5	1.5	2.9	11.0	0.7
Parathion, 1%+ Sulfur	35	27.5	22.8	20.0	48.0	266.8	2.0	3.3	—	
Compound 338, 2%[5]	41	37.5	25.0	36.0	80.0	373.3	2.5	3.5	—	
Sprays:										
0.5 pint Systox[6]	50	93.8	70.8	31.0	101.3	374.0	3.0	3.8	8.1	0.0
1 pint Systox	50	36.3	24.5	7.8	24.0	92.5	0.5	2.0	12.8	0.0
1 quart Systox	50	11.0	7.5	2.0	11.3	29.8	0.0	0.5	—	0.0
Check		540.3	483.8	557.5	501.5	765.0	4.0	4.0	3.3	0.0
L.S.D. 5%		29.3	19.9	48.9	22.4	43.1				
L.S.D. 1%		39.6	26.9	66.1	30.2	58.2				

[1] Mean of two dust applications (April 14, 17); single spray application on April 17.
[2] Mite damage to the foliage was graded as follows: no visible injury 0; trace 1; slight 2; moderate 3; severe 4.
[3] Systox samples harvested May 12; residues determined by Cholinesterase method by Pittsburgh Coke and Chemical Co. Ovotran and 338 samples harvested May 13.
[4] Commercial formulation supplied by Niagra Chemical Co.
[5] 4,4'-dichlorobenzilic acid ethyl ester, Geigy, Inc.
[6] A 48.1 per cent emulsion supplied by the Pittsburgh Agricultural Company.

Table 6.—Results of late spring treatments for strawberry spider mite control. Norfolk, Virginia, May 1, 1952.

Treatment	Amt. per Acre. Lbs. or Gallons	Mites per 25 (0.3 Sq. In.) Leaf Samples — Days After Treatment: 2	5	8	18		
		Number	Number	Number	Number	Per Cent Reduction	Residue ppm[4]
Sprays:							
0.5 pint Systox[1]	50	33.0	63.5	15.0	122.3	75.1	0.0
1 pint Systox[1]	50	7.3	19.3	4.5	9.5	98.1	0.0
Dusts:							
Sulfur	35	21.8	55.8	44.8	82.5	83.2	—
Parathion, 1%	30	17.0	20.5	30.5	141.5	71.2	—
Malathon, 5%	39	7.5	9.5	16.0	45.8	90.7	0.0
NPD, 5%	41[2]	11.0	9.3	11.8	59.3	87.9	1.4
TEPP, 1%	32	13.8	218.3	355.8	429.5	12.4	—
TEPP, 1%	35[3]	—	11.3	45.3	150.0	69.4	—
Check	—	605.8	629.0	669.0	490.5	—	0.0
L.S.D. 5%		37.2	28.7	26.0	30.1		
L.S.D. 1%		50.7	38.9	35.2	40.3		

[1] A 48.1 per cent emulsion supplied by the Pittsburgh Agricultural Co.
[2] tetra-n-propyl dithionopyrophosphate; Dupont.
[3] Average of two applications; plots subdivided and retreated May 5.
[4] Systox samples harvested May 12; residues determined by Cholinesterase method by Pittsburgh Coke and Chemical Co. Malathon and NPD samples harvested May 19.

an acre of strawberries was sprayed in the above manner. At the time of treatment the strawberry plants were actively growing.

The results of these tests as presented in table 4 demonstrate the long term mite control obtainable with Systox. At harvest time, 38 days after spraying, the treated areas showed a mite reduction of 95.4 per cent under the check. This degree of control decreased to 60.1 per cent by the end of the picking season. A yield of 38 quarts per 100 feet of treated row as compared to only 10.5 quarts in an equal area of untreated row clearly illustrates the benefits of good mite control.

Table 7.—Results of dipping strawberry plants in various concentrations of Systox prior to transplating as a means of preventing subsequent mite infestations. Norfolk, Virginia. February 27, 1951.

Systox[1] per 100 Gallons of Water	Mites[2] per 25 (0.3 Sq. In.) Leaf Samples — Days After Treatment: 20	75	
	Number	Number	Per Cent Reduction
1 pint	3	90.5	72.4
1 quart	0	10.0	96.9
2 quarts	0	10.3	96.9
Check	29.5	327.5	—
LSD 5%		48.3	
LSD 1%		67.9	

[1] 32.1 per cent emulsion.
[2] *Tetranychus bimaculatus.*

Early Spring Tests.—On April 14 the dusts listed in table 5 were applied to strawberries growing at Norfolk. Just after completion of dusting, and prior to spraying with Systox, a flash rain of 0.33 inches occurred. Weather conditions remained unfavorable until April 17 at which time the dusts were repeated and a single treatment with Systox made at the levels indicated. Application was by means of a rotary hand duster or single nozzle knapsack-type sprayer operating at 50 lb. pressure. All plots were 50 feet long by three beds wide.

The dusts gave somewhat better results than might normally be expected at this time of year (Table 5). However, the April 17 treatments were made under almost perfect weather conditions, followed by two days of near 80° F. temperature. In addition the plants had begun growing rapidly and the erect new growth facilitated coverage. Parathion plus sulfur, Compound 338, Aramite, sulfur, and

Systox (1 pint and 1 quart) gave excellent results after four days, with a reduction from the check of 91 per cent or better. Ovotran was somewhat slower in action but it is significant to note that areas treated with this material showed better control after 32 days than at 4 days. The above materials maintained good mite control up to mid-harvest (May 19) but all except Systox showed a marked increase in mite populations thereafter. Aramite and 10 per cent Ovotran were superior to the other compounds tested as dusts. One-half pint of Systox was quite effective up to 21 days but did not continue to hold the mites in check. The plant stand was too uneven to justify any definite conclusion on yield other than the fact that the yield from the treated areas was increased approximately 300 per cent above the check. There were no evidences of plant injury from any of the materials.

The degree of mite damage to the strawberry foliage was employed as an additional aid in evaluating the various treatments. In scoring the foliage injury five classes were selected as follows: no visible injury 0; trace 1; slight 2; moderate 3; severe 4. In the May 19 ratings, one-half pint Systox (moderate injury) and the check (severe injury) had the most mite damage. At this time the check was almost completely destroyed and failed to produce any more fruit. By the end of the following week all materials except Systox (1 pint and 1 quart) were showing a moderate or near moderate degree of injury with sulfur, Compound 338 and Systox (0.5 pint) approaching severe injury. The high degree of mite control resulting from the 1 pint and 1 quart applications of Systox is reflected in the low plant injury rating at the completion of harvest (May 26).

The excellent strawberry spider mite control obtained with a commercially prepared 3 per cent Aramite dust (Table 5) is of particular interest since preliminary tests with a 3 per cent formulation blended from wettable powder had given only fair control. On April 28 commercially prepared and home blended 3 per cent Aramite dusts were compared in a field of strawberries heavily infested with *Tetranychus atlanticus*. The Aramite formulations were applied with a power duster at about 35 lbs. per acre. A 25 foot cloth trailer was used to insure better coverage. Counts 15 days after treatment showed a 90.1 per cent mite reduction in the commercial Aramite preparation as compared to a reduction of only 60.8 per cent in the wettable powder blend.

Late Spring Tests.—The spring of 1952 was unusually dry and, consequently, favorable for spider mite development. Shortly before harvest mite infestations on strawberries increased so rapidly and to such an extent that many growers suffered severe crop damage. This was particularly true in the case of those who did not follow a definite control program but delayed treating until noticeable plant injury occurred. It has become increasingly apparent that a definite mite control program must be followed if losses of this kind are to be avoided.

A series of insecticidal treatments was applied May 1 to a field of strawberries heavily infested with *Tetranychus atlanticus*. Mites of all stages were present and there was an average of approximately 100 mite eggs per strawberry leaflet. Methods of treatment and experimental design were similar to the early spring tests. The temperature at the time of treatment was 70° F. with a relative humidity of 50 per cent. The maximum temperature for the next two days was 88° F. and 69° F., respectively. The materials tested and results obtained are presented in table 6.

All materials gave an excellent initial mite kill and, with the exception of the single application of TEPP dust, maintained a high level of control after 8 days. At the termination of this experiment, one pint of Systox gave significantly better results (98.1 per cent reduction) than any other treatment and was the only one without foliage damage. Malathon, NPD and sulfur were next in order of effectiveness. Relatively poor long term control was obtained with parathion, one-half pint Systox and TEPP. The TEPP plots which were retreated after 4 days had a reduction of only 69.4 per cent 14 days later and it appears that three applications of TEPP dust at 4 to 5 day intervals may be necessary to control mites when large numbers of eggs are present.

Observations of commercial treatments with TEPP dust have indicated that this material gives better cold weather results than any other available insecticide. Some

indications of decreased effectiveness were noted if the plants were treated while still wet with dew.

Residues.—Sixty-one different samples of strawberries were submitted for residue analyses in 1951–52. These samples were from treatments made at selected intervals under a wide range of temperature and plant conditions. According to the bioassay technique employed in 1951, Systox appeared to be dissipated rather rapidly at first and then maintained a constant relatively low level in the fruit, usually 0.1 ppm (Table A, B, C). In this connection, a pint of Systox per acre applied to ripening fruit gave a residue of only 0.1 ppm when harvested 8 days later. The 1952 Systox residues were determined by the cholinesterase method. None of the samples (Tables 3, 4, 5, 6) had measurable residues notwithstanding the fact that treatments as high as one pint of Systox per acre were made only 11 days before harvest.

Samples of berries treated with several of the dusts were also analyzed for residues. As indicated in table 5, strawberries from the 5 and 10 per cent Ovotran treatments had residues of 0.4 ppm and 0.7 ppm, respectively, 25 days after the last application. Compound 338 gave a residue of 0.11 ppm over the same period. In the late spring tests (Table 6), samples from the 5 per cent NPD plots had a residue of 1.45 ppm at 18 days while 5 per cent malathon samples showed no residues after this interval. Additional applications of 5 per cent malathon at approximately 40 lbs. per acre resulted in residues of 2.7 ppm and 0.0 ppm after 3 and 10 days, respectively. Ripening berries which were treated with a 3 per cent commercially prepared Aramite dust at about 35 lbs. per acre, picked immediately and extracted in benzene, showed a residue of 0.6 ppm.

Dipping Tests.—Strawberry plants are normally transplanted some time in February or early March in the Norfolk area. These plants do not produce a marketable crop of fruit until approximately 15 months later. In the meantime the new plantings are quite susceptible to mite damage and also to injury by the strawberry root louse. Dipping in rotenone-vegetable oil emulsions gives a fair degree of root louse control but is not very effective against mites. During 1951–52 various concentrations of Systox were tested as a dip. The plants were thoroughly immersed for a period of 30 seconds, then removed and allowed to dry for a period of two hours before planting. The results of one series of tests are presented in table 7.

Systox at the one quart and two quarts per 100 gallons of water concentration was quite effective in keeping mites in check during the critical period following planting. The final records, 75 days after treatment, were taken just before the annual decline in mites occasioned by hot weather and represent the peak infestation. One pint of Systox gave fairly good control although some plants showed severe mite injury. Due to the spacing between plants there was a tendency for heavy infestations to develop on some plants while others nearby were free of mites. Approximately 10 per cent of the plants in the one pint dip were seriously retarded in growth and failed to send out vigorous runners. None of the Systox treatments produced any plant injury.

Based on the results of these tests, dipping strawberry plants in a solution containing one quart of Systox per 100 gallons of water offers a cheap and effective means of increased mite control. When home stock is used and when the root louse is not a problem, spraying the plant beds prior to transplanting is suggested as an alternative measure. Where plants are received from out of state, dipping before shipment might have possibilities since such a procedure would greatly decrease the delay and safety precautions necessary in transplanting.

SUMMARY AND CONCLUSIONS.—A number of acaracides were tested during 1951–52 at the Virginia Truck Experiment Station in an attempt to develop improved measures for mite control on strawberries. Particular emphasis was placed on cool weather treatments since damaging mite infestations often occur in late winter or early spring. For the most part, low soil surface temperatures, cold nights and variable weather conditions have made it exceedingly difficult to obtain adequate early season mite protection.

A total of 33 Systox treatments were compared at concentrations of from 0.25 pint per acre through 2 quarts per acre. Systox was generally effective in con-

trolling *Tetranychus atlanticus* and *T. bimaculatus* over a wide range of temperature and plant conditions. Excellent early season control was obtained with 1 pint of 32.1 per cent emulsion per acre at a temperature of approximately 50° F. when the plants were semi-dormant. However, a combination of low temperature (25° to 28° F.) and dormant plants reduced the relative degree of mite control at dosages below 1 quart of Systox per acre. Treatments approximately one month before harvest with 1 pint or 6 weeks before harvest with 1.5 pints of 48.1 per cent emulsion appear sufficient to insure good mite control through the harvest period. The 0.25 and 0.5 pint concentrations of Systox gave fairly good initial control but only relatively short mite protection.

Parathion-sulfur and parathion-Ovotran were outstanding for their rapidity of action and long term control against the two-spotted mite. Although Ovotran was comparatively slow, it showed excellent residual qualities. Combinations of parathion with sulfur and Ovotran produced significantly better results than any of these materials alone. Ten per cent Ovotran and commercially prepared 3 per cent Aramite gave the best results of any dust in early spring treatments against *Tetranychus atlanticus*. Commercially prepared Aramite was greatly superior to Aramite formulations blended from a wettable powder.

The organic phosphorous dusts were characterized by a high initial kill and limited residual action. It appears that as many as three applications of TEPP dust at 4 to 5 day intervals are necessary to control mites when large numbers of eggs are present.

Sulphenone and dinitro caprylphenylcrotonate gave good control of the two-spotted mite. Compound 338 and NPD showed promise against the strawberry spider mite.

Sixty-one different samples of strawberries were submitted for residue analyses. One pint of Systox per acre applied to ripening fruit showed a residue (bioassay method) of only 0.1 ppm when harvested 8 days later. None of the Systox treatments in 1952 had mesaurable residues as determined by the cholinesterase method. Residues of Ovotran, NPD, and Compound 338 were relatively low 3 to 4 weeks after application. Berries treated with malathon had residues of 2.7 ppm and 0.0 ppm after 3 and 10 days respectively.

Dipping strawberry plants prior to transplanting in a solution containing 1 quart of Systox per 100 gallons of water greatly decreased first season mite damage to new plantings.

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Control of the Black Vine Weevil

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The black vine weevil, *Brachyrhinus sulcatus* (F.), has been known as a pest of cultivated plants since 1934 and has been recorded on as many as 77 host plants (Smith 1932). It is widely distributed in the United States and Canada as well as in Europe and Australia. Its common name was derived from its activities in a number of European countries where it has been a severe pest of grapes. In literature from various parts of the United States and Canada it is often listed among the pests of strawberries (Mills & Evans 1934; Breakey & Webster 1951). However, it is considered one of the less destructive of the strawberry root weevils (Wilcox *et al.* 1934, Smith 1943) and has attracted most attention in this country as a serious pest of nursery and ornamental plants (Schuh & Mote 1948).

In Ohio, black vine weevil injury has been most severe on the roots of *Taxus* in commercial nurseries, but injury has been observed also on rhododendron and azalea. Injury has appeared throughout northern Ohio but particularly in northeastern Ohio where many nurseries are located. The Division of Plant Industry of the Ohio Department of Agriculture listed 165 certified nurserymen in Lake County and 541 in 12 counties of northeastern Ohio in 1952.

With the increased popularity of various species of *Taxus* for ornamental purposes and the corresponding increase in the size and number of *Taxus* plantings in Ohio nurseries, the importance of this insect as an economic pest has increased in recent years. In an occasional nursery, populations developed to the point where hundreds of plants were killed after they had grown to the stage where they were salable as ornamental plants.

Inasmuch as *Taxus* plants are commonly "balled and burlapped" when sold in the fall or spring, the black vine weevil larvae may be carried with the plants to the new location. Heavily infested transplants frequently die without becoming established. In nurseries with a shortage of skilled labor, the task of replacing dead plants becomes a burden and magnifies the damage caused by the insect.

The life history of the black vine weevil in Pennsylvania was reported in detail by Floyd F. Smith (1932). His findings have been corroborated for Ohio. Only one brood occurs each year. The adults appear during June and early July. They feed on the foliage at night but hide during the day, primarily in debris and loose soil under the plants. Eggs are simply dropped to the ground during July and August. The chief damage results from the destruction of roots by larval feeding.

Investigations of the control of this insect on *Taxus* in Ohio were begun in 1949. Biological studies were designed primarily to determine the time when insecticides could be applied effectively. In order to determine the relative number in the adult stage at a given period, the insects that could be found in the soil and in debris under one or more plants were counted and the number in each stage recorded. As indicated in table 1, the period of adult emergence varies somewhat from year to year with the variation in weather conditions.

Table 1.—The relative number of insects in the various stages at different times.

Date	Number of Insects Observed	Per Cent in Each Stage		
		Larvae	Pupae	Adults
June 2, 1949	73	4	45	51
June 7, 1950	50	36	64	0
June 15, 1950	41	12	54	34
June 21, 1950	41	22	20	58
July 20, 1950	98	2	0	98
May 23, 1952	184	34	66	0
June 12, 1952	100	11	38	51
June 16, 1952	100	10	27	63

Control Experiments, 1949.—Experiments in control of the black vine weevil during 1949 were carried on in a nursery in Lake County. The plants used were *Taxus cuspidata capitata* approximately 3 feet in height. Treatments were planned in an attempt to kill the beetles before egg laying began.

Seven treatments were used and each was replicated 5 times. Four replicates of each treatment contained five plants per

plot, but in the fifth replicate each plot consisted of four rows with five plants in each row. Consequently, 40 plants were sprayed with each material. Sprays were applied on June 7 with a small power sprayer. Observations indicated that most of the insects were in the adult stage at that time.

The treatments were evaluated by counting all dead beetles that could be found in each plot 6 days and again 10 days after the sprays were applied. The materials used included lead arsenate, DDT, cryolite, chlordane, parathion and BHC.

A total of 493 dead beetles was found where BHC was used as compared with 61 in the untreated check plots and 205 where chlordane was used. However, the data indicated that the plots were too small to yield reliable data because of the unequal distribution of the insects. Apparently most dead beetles were found in the BHC plots primarily because that insecticide acted more quickly than other materials used. An analysis of variance in the data obtained showed that differences were not great enough to be significant.

The entire planting in which the experimental plots were located consisted of approximately 3 acres and was sprayed with BHC on June 16 and 17 at the rate of 2 pounds of gamma isomer per acre. Observations made during the following week indicated that practically all beetles were killed. However, it was learned later in the summer that beetles continued to emerge after the insecticide had lost its effectiveness. These surviving beetles were able to produce enough offspring to reinfest the planting.

CONTROL EXPERIMENTS, 1950.—Experiments carried on in 1950 (Neiswander 1951) consisted of soil treatments applied in the same *Taxus* planting utilized in 1949. Each of six insecticides was applied on a single plot 8 rows wide and comprising .4 acre. A seventh treatment was applied on two such plots rather widely separated. The insecticide used in each plot was mixed with 80 pounds of a complete fertilizer and was applied between the rows of *Taxus* plants with a small lime spreader. The soil was then cultivated thoroughly in order to mix the insecticide with the soil and to throw some of the poison under the plants.

The treatments were applied on May 31 when the insects were in the larval and pupal stages. On July 27, when most of the insects were in the adult stage, all living beetles that could be found in the debris and 2 or 3 inches of soil under each of five plants in each plot were collected and counted.

After the new brood was established, the larval population on the roots was also studied. This was done with the aid of a soil sampler. A cylinder of soil 4 inches in diameter and 8 inches deep was taken under the branches of a plant and sifted through a screen on to black paper in order to find the larvae. Eight such samples were taken from each plot on October 4 and another series of seven samples was taken from each plot during the following spring. The data obtained in the three population counts are shown in table 2.

The new materials designated as heptachlor, dieldrin and aldrin appeared most effective in each of the three population

Table 3.—Spraying experiments in control of black vine weevil. Sprays applied June 16 and 17, 1952.

TREATMENT NO.	MATERIALS USED PER 100 GALLONS	BEETLES UNDER 30 PLANTS JUNE 23–24, 1952			BEETLES JARRED FROM 10 PLANTS JULY 7
		Alive	Dead	% Dead	
1	Chlordane, 2 lbs. 50% WP	7	64	90.1	3
2	Aldrin, 4 lbs. 20% WP	3	43	93.5	0
3	Dieldrin, 4 lbs. 25% WP	4	43	91.5	0
4	Heptachlor, 4 lbs. 25% WP	2	69	97.2	0
5	DDT, 2 qts. 25% Emul. Conc.	11	50	82.0	3
6	Malathon, 4 lbs. 25% WP	12	63	84.0	26
7	Parathion, 3 lbs. 15% WP	11	41	78.8	7
8	BHC, 4 lbs. 10% WP	11	105	90.5	39
	Check[1]	181	26	12.6	—

[1] These counts were made at points taken at random in a large area that had not yet been treated. No replicated check plots were included in the series.

counts. Chlordane appeared more effective than EPN, BHC or DDT. The data indicated, however, that instead of killing the newly hatched larvae the insecticides were killing the beetles as they crawled through the treated soil.

Table 2.—Soil treatments for the control of the black vine weevil. 1950.

Materials Applied per Acre	Adults July 27	Larvae Oct. 4	Larvae 1951
EPN 300, 10 lbs.	54	9	—[1]
DDT, 25 lbs.	64	19	—[1]
BHC, 2 lbs. gamma isomer	27	16	—[1]
Chlordane, 10 lbs.	8	3	—[1]
Heptachlor, 10 lbs.	1	0	0
Dieldrin, 10 lbs.	1	0	0
Aldrin, 10 lbs.	2	0	0
Chlordane, 10 lbs.	6	3	7

[1] The plants in these plots were removed before the final counts were made.

During the latter part of the season of 1950 and in 1951, observations were made in nurseries where chlordane was used both as a spray on the foliage and as a soil treatment and where DDT was used as a spray. These observations indicated rather definitely that foliage applications were more effective than soil treatments and that the beetles could be killed more readily than the larvae in the soil. For that reason foliage sprays were again tested in 1952.

CONTROL EXPERIMENTS, 1952.—During 1952, investigations in control of the black vine weevil were carried on in a nursery in Lorain County. A block of approximately 4,000 *Taxus* plants varying in height from 1 to 3 feet and including four varieties was employed in spraying experiments. Eight treatments were tested and each was replicated 5 times. Plots were four or more rows wide and contained approximately 100 plants each. Preliminary observations indicated that all varieties were rather heavily infested. Soil samples taken at random in the fall of 1951 indicated a population of 18 larvae per square foot of surface area under the *Taxus* branches.

Sprays were applied on June 16 and 17. As indicated in table 1, approximately 63 per cent of the insects were in the adult stage at that time. A 50 gallon power sprayer was used and the spray applied with a spray gun at a pressure of 200 to 250 pounds. Each plant was sprayed liberally from two opposite sides in order to cover the interior where the insects feed.

One week after the sprays were applied (June 23) counts were made of all living and dead beetles that could be found under six plants near the center of each plot. All beetles on the surface of the soil as well as those in the debris and loose soil under each plant were collected and counted.

On July 7, 3 weeks after the sprays were applied, another count was made of the adult population in each plot. This was done at night when the beetles were feeding on the foliage. A white cloth was spread under a plant and the branches jarred vigorously in order to dislodge the beetles. With the aid of flashlights, the beetles were counted on the cloth. Two plants near the center of each plot were used for this purpose. The data obtained in the two population counts are summarized in table 3.

Inasmuch as larvae and pupae could be found in the soil in small numbers on June 23, it is probable that many of the living beetles found in the sprayed plots at that time were newly emerged insects that had not yet crawled into the plants to feed. The data recorded in table 3 again show that aldrin, dieldrin and heptachlor produced best results. An analysis of variance in the data obtained on July 7 shows that a difference between means of 4.08 is necessary for significance. The first five treatments, therefore, were significantly more effective than treatments 6 and 8.

In an effort to prevent insect damage in the entire planting, the plots that had received either malathon, parathion or BHC were sprayed again on July 9, using aldrin on approximately half of the plots and dieldrin on the remainder. At about the same time the grower sprayed all *Taxus* plants in the nursery that were not included in the experimental area, using aldrin at 2 quarts of 25 per cent emulsifiable concentrate per 100 gallons. This area comprised approximately 5 acres. The grower was anxious to inaugurate control procedures inasmuch as many of the older plants had died during the spring months as a result of weevil injury.

In order to determine the population of

larvae that became established later in the summer, soil samples were examined in the manner used in the experiments carried on in 1950. Two samples were examined from each plot on September 23 and four from each plot were taken on October 15. On October 31, another series of four samples was examined from each of the 25 plots that were sprayed but once. Also a total of 30 samples taken at random from the area sprayed by the grower was examined in the same manner. Altogether 370 soil samples, 4 inches in diameter and 8 inches deep, were examined. From these samples only seven black vine weevil larvae were found and all were taken in plots sprayed only on June 16. Three were in a plot sprayed with DDT, three in a plot sprayed with dieldrin and one in a plot sprayed with aldrin.

When compared with the larval population in the same area the previous year, this represents a control of 98.8 per cent. Inasmuch as the preoviposition period for this insect, as shown by Smith (1934), ranges from 4 to 10 weeks, it is probable that the spray applied June 16 was too early for best results. Since adults began emerging during the first week in June, it is doubtful if any eggs were deposited before July 9 when the last application was made. Practically all insects able to emerge were in the adult stage at that time and probably came in contact with the insecticide quickly. This may explain the fact that no larvae were found in 120 soil samples taken in the areas sprayed July 9 with aldrin or dieldrin.

The larval population counts show that both chlordane and DDT produced excellent results in this series of experiments. However, in the 5 other population counts shown in this paper, aldrin, dieldrin and heptachlor all appeared somewhat more effective.

CONCLUSIONS.—Because of the long preoviposition period, sprays designed to control the black vine weevil may safely be delayed until nearly all of the insects have reached the adult stage. At that time complete control may be obtained with one insecticidal application.

In northern Ohio the optimum time for the application usually occurs during the last week in June or the first week in July. In advanced seasons the former period may be preferable.

Good results have been obtained most consistently with aldrin, dieldrin and heptachlor. Any of these materials may be used at the rate of 1 pound of the toxicant per 100 gallons of water.

The same materials were also effective when applied to the soil at the rate of 10 pounds per acre. However, the soil treatments resulted in killing the adult beetles rather than the larvae in the soil. For that reason foliage applications which serve both as contact and stomach poisons appear more reliable.

DDT and chlordane were effective when used as sprays at the rate of 1 pound of the toxicant per 100 gallons of water but were inadequate when used as soil treatments.

Sprays should be applied liberally in order to cover the interior of each plant where the insects feed and where the insecticide remains effective for a relatively long period of time.

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Varietal Tolerance of Alfalfa to the Meadow Spittlebug[1]

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The meadow spittlebug, *Philaenus leucopthalmus* L. has become an increasingly serious problem of alfalfa and red clover in Indiana during the past six years. A pest that is now general throughout the State, it does a great deal of damage by reducing hay yields. A search for varietal tolerance of alfalfa was first begun in 1950 and has been in progress since that time. This paper reports an study of 26 alfalfa selections.

Methods.—Studies were made in a uniform advanced alfalfa nursery seeded at the Purdue Sand Field in August, 1949, without a companion crop which is the practice on sandy soils in northern Indiana. Varieties, replicated four times, were planted in 5-row plots with the rows spaced seven inches apart. Plots were 22 feet in length. Between plots there was an interval of 21 inches. Replicates placed four deep were separated by alleys in grass sod, 5 feet wide. On three sides, the plot area was bordered by Ranger alfalfa and by a grass roadway on the fourth.

The following factors were studied: adult spittlebug populations in the fall, populations of nymphs in the spring, damage from the insect as evidenced by rosetting or internode shortening of the plant, and the effects of these variables on yield and stand. Adult populations were sampled with a 15-inch sweep net, making five sweeps per plot. Populations of the nymphs were obtained by measuring square-foot samples at random and counting the number of nymphs. Rosetting, or damage, was estimated by using a scale of 1 to 9 (1 to 10 in 1951); a rating of 1 classified the plot with little damage, while 9 showed severe injury. Forage yields were obtained by cutting the plots back to 20 feet and harvesting. Stand counts were made by the method described by Kramer & Davis (1949).

Counts of nymphal populations of the spittlebug were made in May 1951 and 1952. Counts were not made in 1950. In 1950, populations were small. This was the first season following late summer seeding and for this reason few spittlebugs were present since there was little material available the previous summer for oviposition by the adults. However, damage due to the relatively lower population was observed and ratings were made in 1950 as well as 1951 and 1952. Following the winter of 1951, the stands of nine varieties were so greatly reduced that they were discarded for spittlebug studies. Seventeen varieties, although reduced in stand, were uniform enough for continued studies.

Experimental Results.—From the data it will be shown that spittlebug damage as evidenced by rosetting or a shortening of the plant internodes is reflected in a yield loss. Data also suggest that selections of alfalfa exhibit different degrees of attractiveness and tolerance to the spittlebug.

The analysis of variance when applied to spittlebug populations, rosetting damage, and yield, showed significant differences between selections each year for all three variables. Table 1 shows that correlations of spittlebug populations between years, damage between years, yield between years, spittlebugs with damage, damage with yield, and yield with stand were significant in most cases.

Correlation of Yield with Stand.—It may be noted that a significant correlation was not obtained between yield and stand in 1950. This is to be expected since there were no measurable stand differences in 1950. By 1951 the stand of some selections had decreased resulting in measurable differences and a highly significant simple correlation between yield and stand was obtained. A partial correlation independent of damage was also significant. Although a significant simple correlation was obtained in 1952, a partial correlation independent of damage was non-significant. This was due to the fact that nine varieties having poor stands were discarded in 1952 and the remaining 17 varieties, although reduced, approximated uniformity as in 1950.

It may be noted that a highly significant correlation between 1951 and 1952 was obtained for yield, but that correla-

[1] Journal paper 669.

[2] Co-leaders of alfalfa project 510, Departments of Entomology and Agronomy respectively.

tions between 1950 and 1951, or 1950 and 1952 were non-significant even when adjusted for differences in stand. The following explanation seems plausible. Since yield is closely correlated with stand, a correlation could not be expected between 1950 when all stands were 98 per cent to 100 per cent complete and 1951 or 1952 when the stands had been reduced. Since stand reduction followed the same pattern in 1951 and 1952, correlating highly, the yields would be expected to show a highly significant correlation for those years. When adjusted for stand, yields in 1951 and 1952 could not correlate significantly with 1951 because of low damage in 1950 and high damage in 1951 and 1952.

Correlation of Rosetting with Stand.—Simple negative correlations between rosetting and stand were significant for alfalfa selections in both 1951 and 1952. This suggested that damage increased as the stand decreased. However, partial correlations independent of yield were non-significant showing that the correlation previously obtained had been due to the close relationship between yield and stand.

Correlation of Rosetting with Yield.—The correlation between rosetting and yield in 1950 was non-significant and was attributed to the low spittlebug populations and injury encountered the first season, as previously explained. In 1951 and 1952, due to severe infestations, negative correlations that were highly significant were otained with rosetting damage on yield. When correlated independently of stand, correlations remained highly significant.

Correlation of Nymphs with Yield and Damage.—Spittlebug populations did not correlate significantly with yields obtained in 1951, but a highly significant correlation was obtained in 1952. However, the correlation in 1952 became non-significant when a partial correlation with the stand held constant was made. This indicates that some varieties of alfalfa support large spittlebug populations, but have a high degree of tolerance in regard to damage since spittlebugs correlate with damage and damage correlates with yield independently of stand influence.

Attractiveness.—Populations of spittlebug nymphs ranged from an average of 58 to 324 per square foot, depending on the variety of alfalfa. Data on rosetting

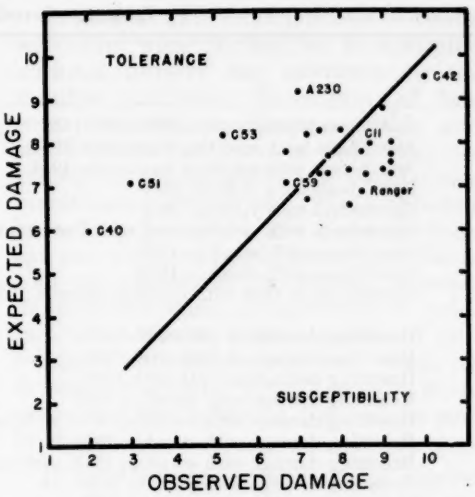

FIG. 1.—The relationship of the observed damage to the calculated expected damage based on the actual spittlebug populations in 1951.

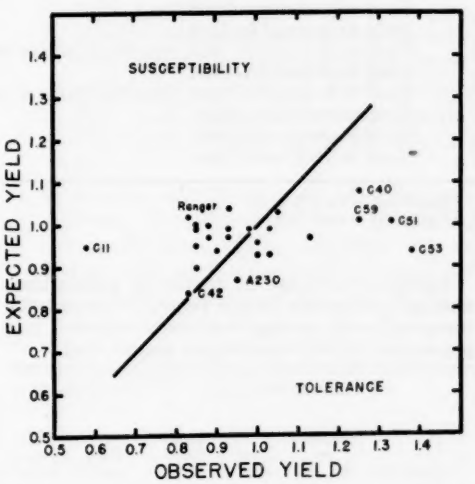

FIG. 2.—The relationship of observed yield to the calculated expected yield based on actual spittlebug populations in 1951.

damage and forage yields were adjusted for all varieties to a spittlebug population of 100 nymphs per square foot. These data for the 17 varieties from which population counts were obtained in both 1951 and 1952 were averaged and are presented in table 2. A study of this table shows that there was considerable difference among the varietal selections as to attractiveness to the spittlebug and ability as a host plant for survival of the insect. The latter does not seem probable. During the 1952 season, population

Table 1.—Correlation coefficients.

	Degrees Freedom	r
Spittlebug populations in 1951 with 1952	15	.649[2]
Spittlebugs with rosetting damage in 1951	24	.418[1]
Spittlebugs with rosetting damage in 1952	15	.602[1]
Spittlebugs with yield in 1951	24	.292
Spittlebugs with yield in 1952	15	.629[1]
Spittlebugs with yield in 1952, stand constant	14	.407
Spittlebugs with stand in 1951	24	.132
Spittlebugs with stand in 1952	15	.522[1]
Spittlebugs in 1952 with stand in autumn 1951	15	.035
Rosetting damage in 1950 with 1951	24	.409[1]
Rosetting damage in 1950 with 1952	15	.438
Rosetting damage in 1951 with 1952	15	.751[2]
Rosetting damage with stand in 1951	24	−.429
Rosetting damage with stand in 1951, yield constant	23	−.290
Rosetting damage with stand in 1952	15	−.668[2]
Rosetting damage with stand in 1952, yield constant	14	−.020
Rosetting damage with yield in 1950	24	−.129
Rosetting damage with yield in 1951	24	−.758[2]
Rosetting damage with yield in 1951, stand constant	23	−.722[2]
Rosetting damage with yield in 1952	15	−.873[2]
Rosetting damage with yield in 1952, stand constant	14	.754[2]
Yield with stand in 1950	24	.217
Yield with stand in 1951	24	.735[2]
Yield with stand in 1951, rosetting damage constant	23	.696[2]
Yield with stand in 1952	15	.757[1]
Yield with stand in 1952, rosetting damage constant	14	.489
Yield in 1951 with 1950	24	.298
Yield in 1952 with 1950	15	.339
Yield in 1952 with 1951	15	.758[2]

[1] Significant at the 5% level.
[2] Significant at the 1% level.

Table 2.—Average of spittlebug populations, damage, and yields for two years, 1951 and 1952, compared with damage and yield adjusted to a population of 100 nymphs per square foot.

	Actual Data			Adjusted Data	
Selection	Av. Nymphs per Sq. Ft.	Damage[1]	Yield Tons/ Acre	Damage[1]	Yield Tons/ Acre
Atlantic	128	8.5	.94	8.1	.97
Washington	103	7.4	1.01	7.4	1.01
87-C10[2]	170	8.2	.97	7.0	1.08
87-C40	61	2.8	1.37	3.5	1.30
87-C41	149	6.7	1.21	6.0	1.26
87-C42	257	9.0	.96	6.6	1.19
87-C49	139	7.3	1.03	6.7	1.13
87-C51	124	4.0	1.36	3.7	1.40
87-C53	158	4.9	1.41	4.3	1.45
87-C59	109	5.7	1.25	5.9	1.24
A202 Syn 4	155	7.8	1.01	7.2	1.05
A204 Syn 4	132	7.2	1.12	6.8	1.15
A220 Syn 3	205	7.3	1.02	5.5	1.20
A225 Syn 1	174	8.2	.95	7.4	1.08
A228 Syn 1	147	8.5	1.05	7.8	1.12
A229 Syn 1	162	6.3	1.12	6.1	1.17
A230 Syn 1	222	6.3	1.01	4.9	1.17

[1] Damage is based on a scale rating, 1–9; the lower the rating, the less the damage.
[2] Key to Coding of Alfalfa Improvement Conference:
C —Prefix for Conference clones.
A —Prefix for Conference synthetics.
87—8, Designates the last number of 1948;
7, Designates open pollinated seed.
Syn 1—Designates first synthetic generation;
Syn 2—Second synthetic generation, etc.

counts were made every ten days on all varieties from the first of May until the end of the month when adults commenced emerging. These data showed 90 per cent survival of the developing nymphs. Possibly the 10 per cent decline by May 28th was due to early emergence of a few adults. There was no difference in the survival of nymphs on different varietal selections. This suggests that the factor of lower populations, particularly evident in selection C40, is due to less attractiveness to the adults for oviposition. Late summer sampling showed no differences in adult spittlebugs between varieties. However, this may not have any bearing on oviposition other than the fact that the infestation was uniform throughout the plot area. The plots are too small to adequately measure a migrating population.

Tolerance.—Table 2 also shows that some varieties such as C40, C51, and C53 are relatively tolerant to spittlebug injury as evidenced by lesser damage and particularly high yields. Varieties such

as C42, A220, and A230 support exceptionally large populations of spittlebugs resulting in high damage and low yields as might be expected, while others such as Atlantic are comparably intolerant with heavy damage and low yield from moderate to average populations. Based on 2 years of damage data (1950 and 1951) and 1 year of spittlebug population data, Ranger, Buffalo, Grimm, and C11 which are not averaged in the table due to stand loss in 1952, were in the non-tolerant classification for the period studied.

Since all 26 varietal selections were studied in 1951 and the data obtained in 1952 correlated with 1951, 1951 data were used in the graphic presentation of figures 1 and 2. In figure 1, the expected damage, Y, using the regression equation, $Y=5.268+.0131X$, has been calculated from actual spittlebug populations and plotted against observed rosetting. Varieties falling close to the line reacted as would be expected, while the extreme deviations, particularly of C40, C51, and C53 above the line suggest tolerance. In figure 2, the expected yield, Y, using the regression equation, $Y=1.136-.0009X$, was likewise calculated and plotted against the observed yield. The line on the graph indicates the division between susceptibility and tolerance; in this case, the varieties showing tolerance being below the line. C53 and C51 are again outstanding, but C59 ranks higher, although in figure 2 it more closely approximated the line. This suggests that comparable visible damage may have less effect on yield in the case of C59.

SUMMARY AND CONCLUSIONS.—Alfalfa selections were studied in a replicated uniform nursery for tolerance to the meadow spittlebug. Three years of data were obtained on damage, yield, and stand; while two years of data were obtained on actual spittlebug populations. Significant correlations were obtained between populations and damage, damage and yield, and yield and stand. There was no significant correlation between damage and stand, independent of yield; but there was a highly significant correlation between damage and yield, independent of stand. From the data, it appears that the following conclusions may be made:

1. Alfalfa may be selected for degrees of resistance or attractiveness to the spittlebug as shown by selection C40 supporting low populations in contrast to C42, A220, and A230 with very high populations.
2. Alfalfa, although attractive to large spittlebug populations, may be selected for tolerance. Data suggest that C53, C51, C40, and possibly C59 have a high degree of this factor.
3. Alfalfa selections may also be relatively intolerant to low spittlebug populations. This appears to be the case with selection C11 and the following varieties: Ranger, Buffalo, Atlantic, and Washington. To a lesser extent, this may be the case with Grimm and Stafford.

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SIRDAR MOHAN SINGH

Announcement of the sudden death on November 6, 1952, of Sirdar Mohan Singh of the Directorate of Plant Protection, Quarantine and Storage, New Delhi, India, came as a shock since he had only about a month previously completed a nine months' study-tour in the United States under the Point 4 Program. While in the United States he visited many laboratories of the Bureau of Entomology and Plant Quarantine and State Experiment Stations, and thus became widely known. Mr. Singh was born at Amritsar, Punjab, May 15, 1910.

Upon receiving the M.S. degree with honors, from the Punjab University, he joined the Indian Agricultural Research Institute as a research assistant. In 1944, he was appointed Assistant Director of Storage in the Ministry of Food and was soon promoted to Deputy Director of Storage. In 1947, he was transferred to the Directorate of Plant Protection Quarantine and Storage as Storage Entomologist. He was also Assistant Director of Locust Control. He was author of numerous articles mainly dealing with household and storage pests. One of his last contributions was a beautifully illustrated bulletin, published jointly with Hem Singh Pruthi, "Pests of Stored Grain and Their Control."

FRED C. BISHOPP

Varietal Tolerance of Alfalfa to the Potato Leafhopper

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Entomologists and plant breeders have recognized the importance of finding selections less attractive or more tolerant to insect attacks. Snelling (1941) has reviewed the literature and tabulated records of resistance in nearly 100 plant species in which over 100 insect species are involved.

The potato leafhopper, *Empoasca fabae* Harris, has long been known as an important pest of alfalfa. Poos & Johnson (1936) reported the injury was directly proportional to the number of leafhoppers attacking the plants. Granovsky (1928) caged healthy alfalfa plants in the field and greenhouse with leafhoppers. Prolonged feeding resulted in badly discolored and stunted plants while young plants were often killed outright. The root system was found to be weakened. Differences among alfalfa varieties in susceptibility to attack by leafhopper populations have been reported by Jewett (1929), Searles (1932), Farrar & Woodworth (1939) and Graber (1941).

Data showing the relationship between the number of leafhoppers and plant discoloration or damage with subsequent effects on stand and yield are presented in this paper.

MATERIALS AND METHODS.—In addition to yield and stand data, leafhopper population counts were made in a Uniform Advanced Alfalfa Nursery located at the Sand Experimental Field in northern Indiana. This nursery consisted of 26 selections and varieties planted in 5-row plots 22 feet long with 7 inches between rows and 21 inches between plots. It was seeded in August 1949 with four replications in a randomized block. The nursery was surrounded on three sides by Ranger alfalfa and on the fourth side by a sod roadway. The counts were obtained by making 5 sweeps in each plot with a 15-inch net except in 1951 when the plots were sampled with 6-inch embroidery hoops. Compared with the larger samples obtained by conventional sweepings this technique was considered unsatisfactory because of extreme variablity in sampling and data are considered unreliable even though certain trends appeared to exist. Hence, no data on leafhopper populations for 1951 are included. The dates of sampling leafhopper populations were as follows: July 29, 1950; July 1, 1952 (second harvest); and Sept. 4, 1952 (third harvest).

Table 1.—F values from the analyses of variance for leafhopper counts, damage, and forage yield.

		F VALUE FOR VARIETIES			
		Leafhoppers		Yield	
YEAR	HARVEST	Number	Damage	Unadjusted	Adjusted for Stand
1950	2	1.66[3]	6.13[4]	14.51[4]	[2]
1950	3	—	—	3.83[4]	[2]
1951	2	—	—	3.97[4]	1.62
1951	3 (early)	—	14.03[4]	—	—
1951	3 (late)	—	33.37[4]	1.60	—
1952	2	1.83[3]	4.00[4]	2.13[4]	1.14
1952[1]	2	2.79[4]	—	—	—
1952	3 (early)	—	17.41[4]	—	—
1952	3 (late)	3.69[4]	5.50[4]	2.56[4]	1.00

[1] Nymphs only.
[2] No stand difference.
[3] Significant at the 5% level.
[4] Significant at the 1% level.

Table 2.—Per cent of a total stand present following winter killing.

	PER CENT STAND		
SELECTION[1]	1950	1951	1952
Ranger	90	80	54
Buffalo	98	80	53
Grimm	98	80	46
Atlantic	99	86	53
Stafford	99	94	52
Washington	98	94	77
A201 Syn 4	96	84	46
A202 Syn 4	98	90	74
A204 Syn 4	98	93	78
A206 Syn 4	96	89	57
A207 Syn 4	97	82	59
A220 Syn 4	96	87	55
A225 Syn 1	97	83	68
A228 Syn 1	97	85	67
A229 Syn 1	97	92	79
A230 Syn 1	95	87	74
87-C10	99	96	69
87-C11	98	57	38
87-C19	98	89	70
87-C40	99	98	89
87-C41	100	98	90
87-C42	99	96	77
87-C49	98	92	67
87-C51	98	97	81
87-C53	99	97	82
87-C59	99	95	84

[1] "C" designates selections of the Alfalfa Improvement Conference. 87 designates open-pollinated seed produced in 1948. The "A" numbers are conference synthetics.

[1] Co-leaders of alfalfa project 510, Departments of Agronomy and Entomology respectively. The authors express their appreciation to R. T. Everly for his interest and suggestions in this study.

Along with the counts each plot was rated for color of forage. This rating was used as a measure of damage. The *1* to *9* scale adopted by the Alfalfa Improvement Conference was used. The best or dark green plots were rated *1* and the poorest or most yellowed plots were rated *9*. Damage ratings were made on the following dates: July 23, 1950, Aug. 23, 1951; Sept. 12, 1951; July 14, 1952; Aug. 25, 1952; and Sept 4, 1952.

The percentage stand was determined by counting the 6-inch units missing in the 5-row plots and converting the units missing to per cent stand loss as described by Kramer & Davis (1949).

Since the leafhoppers are not a problem on the first crop, only data from the second and third harvests were used in analysis. Yields are expressed in tons per acre at 12 per cent moisture.

EXPERIMENTAL RESULTS.—The F values from the analyses of variance for leafhopper numbers, damage ratings, and yield are given in table 1. The one sampling in 1950 on the second harvest and the two samplings in 1952 showed varietal differences in leafhopper populations. This was also true of the damage in all three years. Yield differences between varieties were significant in 1950, 1951, and 1952 for both second and third harvests with one exception. In 1951, the month of August was very dry, thereby masking any potential yield differences.

Correlation of Yield with Stand.—The yields, second harvest in 1951, and second and third harvests in 1952, were correlated with stand. Positive correlation coefficients with 24 degrees of freedom were 0.821, 0.850, and 0.882 respectively for the variety component. Hence, approximately 70 per cent of the yield difference was associated with stand. When the the yields were adjusted for stand and analyzed the differences exhibited prior to adjustment ceased to exist. There were no measurable (Table 2) stand differences in 1950. However, the yield differences were significant even though not associated with stand.

Correlation of Damage with Stand.—Data suggested that the leafhopper damage as measured by color was associated with stand differences rather than with yield *per se*. Three facts corroborate this:

1. Yields adjusted for stand differences were not significantly different.

Table 3.—Correlation coefficients.

VARIABLES CORRELATED	df	r
Damage		
1950 and stand 1951	23	−.381
1951 and stand 1951	23	−.572[2]
1951 and stand 1952	23	−.655[2]
1952 and stand 1952	23	−.449[1]
1950 (2nd late) and 1951 (3rd early)[3]	23	+.782[2]
1951 (3rd early) and 1951 (3rd late)	23	+.721[2]
1951 (3rd early) and 1952 (2nd late)	23	+.611[2]
1951 (3rd early) and 1952 (3rd early)	23	+.869[2]
1951 (3rd late) and 1952 (3rd late)	23	+.717[2]
1952 (2nd late) and 1952 (3rd late)	23	+.631[2]
1952 (3rd early) and 1951 (3rd late)	23	+.883[2]
Leafhoppers		
in 1950 and 7/1/52	18	+.554[1]
in 1950 and 9/4/52	18	+.247
in 7/1/52 and 9/4/52	18	+.279
in 1950 and damage 1950	24	+.386
in 1952 (2nd) and damage 1952 (2nd)	18	+.615[2]
in 1952 (3rd) and damage 1952 (3rd)	18	+.593[2]

[1] Significant at the 5% level.
[2] Significant at the 1% level.
[3] Numbers in parenthesis refer to harvest sampled.

Table 4.—Varietal attractiveness and tolerance of alfalfa to the potato leafhopper.

SELECTION	ATTRACTIVENESS Index[1]	OBSERVED DAMAGE Index[2]	TOLERANCE Index	TOLERANCE Leafhoppers[3]
Ranger	9.0	8.6	2.5	188
Buffalo	3.0	7.8	8.0	111
Stafford	5.5	7.8	6.0	140
Washington	7.0	8.4	5.5	149
A202 Syn 4	4.0	6.4	6.5	134
A204 Syn 4	8.5	6.0	1.0	205
A206 Syn 4	2.0	4.6	6.5	135
A207 Syn 4	2.0	6.2	9.0	106
A225 Syn 1	5.5	5.6	5.5	161
A228 Syn 1	1.0	3.8	7.5	120
A229 Syn 1	5.0	4.5	3.5	176
A230 Syn 1	4.0	6.1	5.8	145
87—C19	2.0	4.9	8.0	113
87—C40	5.0	3.3	2.8	185
87—C41	4.0	5.7	6.5	135
87—C42	2.0	3.9	7.0	133
87—C49	4.8	6.4	6.0	140
87—C51	3.0	3.7	5.5	147
87—C53	2.5	3.3	6.0	142
87—C59	6.0	5.9	4.0	165

[1] Indexes based on scale of *1–9* used by the Alfalfa Improvement Conference. A rating of *1* indicates low attractiveness, low damage, or high tolerance.
[2] Damage index based on 3 year average of observed damage on four harvests.
[3] Population per five sweeps producing average damage of *5.0*.

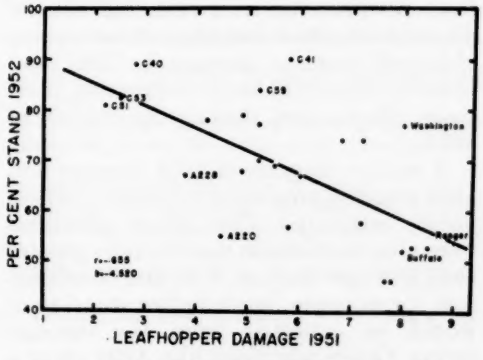

FIG. 1.—The relationship of per cent stand in 1952 to leafhopper damage in 1951.

2. Partial correlations for 1951 and 1952 between damage ratings and yield with stand held constant were not significant.
3. The correlations between damage ratings and stand were significant (Table 3).

Damage ratings in 1950 did not correlate significantly with stand in 1951. This was probably due to the limited range in stand differences. In all other cases damage ratings and stand counts were significantly correlated.

There was an excellent stand in all plots in 1950 (Table 2). However, by 1951 Ranger & Buffalo, the two wilt resistant varieties, and Grimm, a winter hardy variety, had decreased in stand to 80 per cent. The stand of a majority of the selections with "C" numbers and a few of the "A" numbers (synthetics) was better than 90 per cent. After the third winter the stand of Ranger and Buffalo was down to 54 and 53 per cent respectively. Grimm, winter hardy but wilt susceptible, decreased more, probably as a result of a wilt infection evident in parts of the nursery. C40, C41, C51, C53, and C59 maintained good stands. Generally, the selections with stands above 80 per cent had exhibited comparatively little damage when rated the previous two seasons. The per cent stand of C11 decreased to a point that no further data were obtained on insect populations and color.

Possibly, the intense discoloration caused by the leafhopper infestations resulted in decreased photosynthetic action causing a decreased storage of food reserves in the roots with a subsequent decrease in stand. Graber *et al.* (1927) have stressed the necessity of adequate food reserves for overwintering plants. The highest stand-damage correlation was obtained between damage in 1951 and stand in 1952, (Table 3) suggesting that more plants died during the winter of 1951.

A scatter diagram of 1951 damage and 1952 stand is presented in figure 1. These values suggested that some selections were able to maintain stands under greater than average damage. C41 and Washington, for example, has a better stand than would be expected from the damage rating. Other selections like A220 show a per cent stand below that expected. Part of this deviation from expected may be due to inherent differences in plant color rather than a reflection of insect damage.

Correlation of Damage with Leafhopper Populations.—The correlation coefficients between the various damage ratings are presented in table 3. These significant correlation coefficients indicated the selections reacted similarly from year to year to leafhopper infestations. There was a significant relation between early damage symptoms and ratings made late just prior to harvesting. These data suggested that such damage evaluations could be taken any time during the season when the leafhopper population was large enough to cause discoloration of the plant.

The correlation of leafhopper populations in 1950 with counts made in 1952 on July 21 was the only correlation of leafhoppers between years that was statistically significant (Table 3). In 1950 the sampling was at a time when the adult population was low. On July 21, 1952 the nymphal population was counted separately from the adults and used in the calculations. Since both samples were mostly nymphs and since the nymphs do not move from plant to plant as readily as the adults, a less variable sample was obtained. The samples consisting of adults were so highly variable the correlation coefficients were non-significant.

Table 3 also shows that when leafhopper populations were correlated with damage, two out of three correlations were significant. It is possible that a significant correlation might not be obtained because of tolerance.

Correlations between leafhopper populations and stand were not significant. This was probably due either to migration of the insect resulting in considerable plot to plot variation, or to varietal tolerance.

Attractiveness and Tolerance.—From an examination of table 4 it appears that differences in attractiveness and tolerance of the alfalfa selections to the potato leafhopper existed. Column 1 represents the attractiveness of the selections to leafhopper populations in this study. For easy comparison, data have been converted to a scale rating of *1* to *9* where *1* represents the selection having the lowest population or an average of 93 leafhop-

pers per five sweeps for A228. A rating of *9* represents the other extreme, in this case, Ranger, which received an average count of 226. These ratings are directly proportioned to actual population counts and are based on an average for all sampling dates, 1950–1952.

Column 2, represents the actual damage rated in the field and is an average of three years on five different harvests.

Column 3 indicates the relative tolerance of the various selections studied. These ratings were obtained as follows: Using the regression equation of leafhopper populations on damage index, the average leafhopper populations for each variety on all dates of sampling were adjusted to the population required for each selection to effect on average damage rating of *5*. These adjusted population values were then converted to the scale of *1* to *9* as previously described. In this case a rating of *1* indicative of the highest tolerance among the selections was represented by A204 requiring a population of 205 leafhoppers per five sweeps to effect average damage, while A207 or A220 were rated *9*, showing least tolerance since they recieved the same amount of damage from a lesser population of 106 leafhoppers per five sweeps.

It is apparent that some selections are less attractive than others. However, this unattractiveness is not always associated with green color. Some selections, Buffalo, C19, and 207, show a high damage rating with low populations. Conversely, Ranger shows relatively high tolerance but it is severely damaged because it attracts such high populations. Other selections exhibiting varying degrees of tolerance are C40, A229, and A204. Selections C51, C53, and C42 are very promising. Even though they do not show tolerance, they have attracted comparably low infestations which have produced little damage.

Unfortunately it was necessary to exclude five selections: Grimm, Atlantic, A201, A220, C10, and C11 from table 4. These selections were excluded from the latter part of the study because of particularly high stand loss in some replicates. An average of data from two harvests showed all of these selections with the exception of C10 to rank high on the susceptible scale. Data suggested a relatively high degree of tolerance from C10 for the two harvests.

SUMMARY AND CONCLUSIONS.—Populations of the potato leafhopper, damage as measured by plant discoloration, hay yields, and persistence of stand were studied during 1950, 1951, and 1952 in a replicated advanced Uniform Alfalfa Nursery in a search for tolerance to the insect.

Data indicated that alfalfa selections may be unattractive or tolerant to leafhopper infestations. C40, C59, C10, and A204 exhibited tolerance to leafhopper populations; others, including A206 and A228 had low damage ratings associated with low populations suggesting unattractiveness.

Yield differences ceased to exist when corrected for stand differences. Seventy per cent of the yield differences were associated with stand differences. The data suggested that leafhopper damage was associated with stand reduction rather than yield *per se* since partial correlations between damage and yield with stand held constant were not significant. Also there was a significant correlation between stand and damage.

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Failure of Residual Insecticides to Control House Flies[1]

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A program of barn spraying for house fly control was set up in 1949 to study the effects of certain insecticides on the fly population and particularly, to see how long it would take for the development of resistant strains so that we could recommend the most promising residual insecticides for fly control. These studies were initiated following the experience of 1948 (Hansen *et al.*, 1949) when DDT resistant flies were found in a number of areas in New Jersey. Studies in the 1949 season were reported by Hansens (1950). Since then studies of lindane, methoxychlor, chlordane and dieldrin in the same barns have continued until 1952 when they failed to give control. During this period we have recommended lindane and methoxychlor for use in dairy barns because they kill flies faster and have greater safety than chlordane and dieldrin.

The barns selected for these studies were of various constructions, some wood and others cinder block. In all sanitation was reasonably good, and consequently fly populations were considered average for New Jersey dairy barns, but varied greatly from season to season depending on weather conditions. Gutters were cleaned daily or every other day and manure was usually removed to fields at least twice a week. The main source of breeding on these farms was within box stables and calf pens which sometimes were not thoroughly cleaned for 2 weeks to a month.

Spray applications were made to all ceilings, walls, and stanchions using a single nozzle or a three nozzle broom and a power sprayer operating at 125 to 150 pounds pressure. One gallon of spray was applied to about 400 square feet of surface. Care was always taken to cover feed troughs, watering cups, and feed before spraying.

Each year spraying was started when flies became numerous about the middle of June and as often as necessary thereafter. In practice flies were usually abundant for about a week before time was available for respraying. Another application was deemed necessary when a fly index of three was reached in a barn. This index was obtained by averaging a visual observation of the number of flies and actual counts of flies resting on three stanchions. The following scale was used in determining the fly index:

1. Scarce—total flies in barn easily counted,
2. Few—1 fly resting on each stanchion,
3. Numerous—two to five flies per stanchion, and
4. Abundant—more than five flies per stanchion.

These studies were carried through into 1952 in 9 barns applying insecticides per 100 gallons of spray as follows: barns 1 and 5, 8 pounds of 25 per cent lindane wettable powder; barns 2 and 7, 5 quarts of 20 per cent lindane emulsion; barns 9 and 10, 40 pounds of 50 per cent methoxychlor wettable powder; barns 8 and 11, 20 pounds of 50 per cent wettable chlordane; and barn, 13, 8 pounds of 25 per cent wettable dieldrin (1952, 4 pounds of 50 per cent wettable dieldrin). In barns 1 and 2 applications of lindane were started in August, 1949, after DDT failed to give control of the fly population which was found to be resistant to this insecticide in both locations. Barn 8 was sprayed with lindane in 1949 and chlordane applications were started in the following year. Data concerning the number of applications in each barn and the results are set forth in table 1.

From this table it is evident that there was a wide range in effectiveness of sprays of all materials. The longer periods of control were obtained early in the season when flies were less numerous, especially in the years 1950 and 1951 when breeding conditions were generally unfavorable to house flies until late July.

In 1952 the first application of methoxychlor, dieldrin and chlordane gave unsatisfactory fly control and lindane performed poorer than in previous years. With the second application all materials failed to give control. In the case of methoxychlor, it should be noted that the last application in 1951 also gave poor results, and it appeared that resistant flies

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey ,Department of Entomology.

were present. Development of resistant strains was not recognized in barns sprayed with the other materials. It now appears, however, that there were some resistant flies in all barns even though their numbers were too few to make an impression on the investigator.

Following the initial failure of all materials in 1952, we turned to alternate materials. Accordingly, barns sprayed since 1949 with lindane were sprayed with methoxychlor and those treated with methoxychlor, chlordane, and dieldrin were treated with lindane except that one chlordane treated barn was sprayed with a synergized pyrethrum formulation. With all of these treatments little fly control resulted. Subsequently, other materials were tested. Lindane plus synergist, sprayed as a residual and lindane plus TEPP and syrup sprinkled on floors failed to give more than immediate kills of flies. In the one barn where it was tried, 32 pounds of 25 per cent wettable dilan per 100 gallons of spray gave three weeks of control. This was the only commercially available material to give at all satisfactory fly control.

With the failure of control, the question arose whether there were resistant flies in all of these barns or the very favorable breeding conditions in 1952 were resulting in extremely large fly populations and consequent lack of control. Accordingly, before any of the above mentioned alternate materials were used in the barns, it was decided to make some laboratory studies to make sure we were dealing with resistant flies. Flies were collected in seven of the barns and brought to the laboratory where tests were made on residues on plywood panels. Flies were exposed for the desired period in embroidery hoop cages according to the technique of Barber & Schmitt (1948).

Table 2.—Laboratory tests on flies collected in barns where control with residual insecticides failed.

Barn	Insecticide on Treated Panel	Minutes Flies Exposed	Total Flies Exposed	Per Cent Flies Killed
1	lindane[1]	15	48	0
	methoxychlor	15	67	0
	DDT	15	34	0
2	lindane[1]	15	50	0
	methoxychlor	15	55	3.6
	DDT	15	28	3.6
9	methoxychlor[1]	15	74	4.1
	lindane	5	51	10.2
	DDT	15	41	7.3
10	methoxychlor[1]	15	28	3.6
	lindane	5	34	2.9
	DDT	15	27	7.4
8	chlordane[1]	15	46	2.2
	DDT	15	29	0
11	chlordane[1]	15	20	5.0
	DDT	15	10	10.0
13	dieldrin[1]	15	24	4.2
	DDT	15	33	0
Non-resistant strain				
	lindane	5	30	100
	methoxychlor	15	37	100
	DDT	15	25	84
	chlordane	15	29	100
	dieldrin	15	24	100
	untreated	—	26	4.0

[1] Insecticide used in the barn 1949 to 1952.

Table 1.—Treatment of barns for house fly control for 4 years and resulting control.

Barn	Treatment	No. of Applications 1949	1950	1951	1952	Days Control 1949 to 1951	Spray per Application 1952 1st	2nd
1	lindane wettable	1	3	2	2	28 to 56	28	4[1]
5	lindane wettable	3	3	2	2	28 to 56	18	7[1]
2	lindane emulsion	1	3	3	2	18 to 35	19	7[1]
7	lindane emulsion	3	3	2	2	28 to 55	19	10
9	methoxychlor wettable	3	3	3	2	7 to 42	4	4[1]
10	methoxychlor wettable	3	3	3	2	14 to 43	9	4[1]
8	chlordane wettable		2	3	2	15 to 42	4	7
11	chlordane wettable	3	3	2	2	20 to 49	12	4[1]
13	dieldrin wettable	2	3	2	3	15 to 57	4[1]	2[1]

[1] First time counts were made after spraying.

Exposures were made to DDT and methoxychlor, applied to panels at 1.0 mg. per square inch and to lindane, chlordane, and dieldrin at 0.1 mg. per square inch. All insecticides were applied in acetone solution. A laboratory strain (Wilson strain) was used for comparison in these tests. Results of the first series of tests are set forth in table 2.

These data show that flies from all of the barns showed marked resistance to the insecticide used in the barn, to the best alternate material and to DDT. There were differences between strains from various barns, but they were not great when the kill of these strains is compared to the much higher mortality of the non-resistant laboratory strain.

In order to find out something concerning the magnitude of this resistance, flies from four of the treated barns were then exposed for 1 and 2 hours to deposits of five residual insecticides as shown in table 3.

From this table it is evident that all strains showed very appreciable resistance to all materials and that the most highly resistant strain to all of the insecticides

Table 3.—Laboratory tests of resistant flies collected in four barns on residual deposits of insecticides.

Insecticide	Exposure on Panel	Per Cent Mortality After 24 Hours			
		Barn 1	Barn 2	Barn 9	Barn 13
Lindane	1 hour	0	5.9	5.3	0
0.1 mg./sq. in.	2 hours	9.6	15.4	9.7	0
Methoxychlor	1 hour	2.0	0	8.3	7.1
1.0 mg./sq. in.	2 hours	6.1	16.7	6.6	0
DDT	1 hour	4.8	8.7	0	0
1.0 mg./sq. in.	2 hours	3.8	0	7.4	0
Chlordane	1 hour	2.6	0	0	0
0.1 mg./sq. in.	2 hours	2.9	6.4	18.2	0
Dieldrin	1 hour	6.7	0	1	2.9
0.1 mg./sq. in.	2 hours	18.0	6.9	23.8	0

Insecticides used in barn—lindane, lindane methoxychlor, dieldrin.

had been produced in barn 13 sprayed 10 times in 4 years with dieldrin. It is noteworthy that so far as we know no other dieldrin had been used in the vicinity of this barn or on neighboring farms. The second highest resistance was shown by flies from barn 1. Sanitation in these two barns was slightly better than that in barns 2 and 9, and it is likely that the selection of resistant individuals was more severe in barns 1 and 13. Consequently a more resistant population resulted.

These studies seem to show widespread development of resistant strains in New Jersey which can withstand all of the chlorinated hydrocarbon insecticides even though a single material has been used in a more or less isolated dairy barn for several years. It is interesting to speculate whether it is significant that widespread resistance to DDT appeared in a year favorable to fly breeding and resistance to these other insecticides similarly developed in 1952, a year extremely favorable to flies.

With the failure of lindane, methoxychlor, chlordane and dieldrin to give control in dairy barns where they had been used for more than 3 years, with the failure of alternative materials to give control in these barns, and further with proof in the laboratory that flies from these barns were resistant to all available chlorinated hydrocarbon insecticides, it became necessary to recommend use of space sprays in all barns included in these studies.

SUMMARY.—From 1949 to 1952 wettable powders of lindane, methoxychlor, chlordane and dieldrin and lindane emulsion applied as residual sprays to walls and ceilings of dairy barns gave house fly control for a month or more after each application depending on fly breeding conditions. Of these materials, lindane and methoxychlor were recommended for use in dairy barns because of their faster killing action and greater safety. In 1952 all of these materials failed to give control and the best alternate material also did not give control. Laboratory studies showed resistance of seven strains of flies to the insecticide used in the barn from which the strain was collected, the alternate material tested in that barn, and to DDT. Extensive tests with fly strains from four of these barns showed the flies were very resistant to all five insecticides.

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Survey and Control Studies of Beetles Attacking Windthrown Trees in the Adirondacks

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On November 25, 1950, a severe windstorm caused widespread damage on 343,000 acres in the Adirondack forests of New York State, resulting in partial to complete blowdown, particularly of red spruce, comprising some half billion board feet. In addition, countless numbers of trees, although still standing, were rendered more susceptible to insect attack and to other unfavorable conditions.

That this extensive blowdown would result in the invasion of storm-felled trees by bark beetles and borers was a foregone conclusion, and it was desirable, especially in view of contempleted salvage operations, to know what beetles were present and attacking, and, as salvage progressed, to know whether the operations were keeping ahead of the attacks of the more serious insect invaders.

A second, and in some ways potentially more important, question was whether conditions had developed or might develop which would favor the build-up of eastern spruce beetles, *Dendroctonus piceaperda* Hopk., to such an extent that live standing trees would be subject to and succumb to beetle attack.

In connection with both of these questions, it was desired to know whether spraying, especially from the air, would have any effect in preventing or minimizing invasion by any of the anticipated insects.

Organization.—In the project which was set up to make the studies reported in this paper, the office of the State Entomologist of the New York State Science Service furnished the research personnel; the Bureau of Forest Pest Control of the New York State Conservation Department with the cooperation of other units of the Division of Lands and Forests, especially those stationed in the forest districts involved, furnished personnel and materials for laying out field plots, and for assisting in making field observations, setting up experiments, spraying, and taking data. The New York State Museum was responsible for the identification of the insects collected.

Science Service temporary personnel assigned to the project, who spent full time in the field were Mr. Robert K. Bennett in the summer of 1951 and Mr. Robert Campbell and Mr. Roger Ryan, in the summer of 1952. In addition, Professor A. H. MacAndrews made available insectary facilities at the New York State College of Forestry in Syracuse.

Procedure.—*Study Plots.*—Typical blowdown areas were located in the vicinity of Stratford, N. Y., northeast of Little Falls. Three study plots were laid out, representing different soil, moisture and exposure conditions from swamp to dry, well-drained hilltop. They ranged in size from 5 to 20 acres. Each study plot was also a spray plot, and to it was assigned a nearby check plot of similar composition.

In each plot, study trees were chosen, numbered and marked for regular routine observations. They represented all conditions of mature and over-mature red spruce from trees broken off at the stump and trees on the ground but still attached to their roots, to "leaners" at various degrees from the vertical. The diameters were of a "merchantable" size from the point of view of salvage.

Aerial Spray Experiments.—In 1951 two of the study plots were sprayed by helicopter with DDT, and the third was divided so that one-half was sprayed with DDT and the other with lindane.

In 1952 the same plots were sprayed, but with a Stearman airplane instead of a helicopter, and using benzene hexachloride on the plot that had been sprayed with lindane in 1951.

The DDT solutions were made up by adding 1 pound of technical DDT to 1 quart of Sovacide (PD544B) and diluting with kerosene to make 1 gallon of finished spray.

The lindane solution was made up by diluting 20 per cent lindane concentrate to 1.25 per cent lindane with kerosene. The BHC spray was formulated by dissolving 36 per cent γ BHC in Velsicol AR-60 and diluting with kerosene.

[1] N. Y. State Science Service, State Education Department.
[2] Bureau of Forest Pest Control, N. Y. State Conservation Department.
[3] N. Y. State Museum, State Education Department.

The Bell helicopter was equipped with a 20-foot spray boom on which 39 spray nozzles were mounted at 6-inch intervals. For the first spray, B-2 nozzles were used, with a nozzle pressure of 35 psi., and air speed averaging 35 m.p.h. The second and third sprays were applied through B-1 nozzles at 20 psi.

The Stearman plane was equipped with 30-foot boom on which 42 B-5 spray nozzles were mounted at 6-inch intervals. The pressure at the nozzles with the plane traveling at about 80 m.p.h. was 70 psi.

In order to make observations on the amount of spray penetration through the forest canopy, and on coverage, 4 by 6 inch glass plates were placed at intervals in each plot, and the sites connected by string lines for guidance. These same string lines were used as guides to the study trees. Five to twenty-four plates, depending on the size of the plot, were placed in each plot before each spray application, on the tops of the boles of down trees, on leaners with various degrees of open and closed canopies above, in the crowns of down trees on the stem portion, and tied to the undersides of the boles of down trees and leaners. After each spray was applied, the plates were collected, the spray droplets were counted, and spray distribution determined.

Because of the variation in density of the forest canopy, the spray droplet distribution was not even. However, the plates did serve to indicate the relative distribution of the sprays over the plots. Better distribution was obtained by helicopter than by airplane. The reason is probably due to the fact that the helicopter flew more slowly and the down draft from the rotor directed the spray toward the canopy.

Although it was recognized from the start that aerial spraying of blowdown timber had a limited chance of success, it was thought that since the helicopter would be capable of flying closer to the timber and flying more slowly, it would be able to make better application of spray than the airplane. As it resulted, because of rough terrain and numerous stickups in the blowdown, the helicopter had to fly higher and faster than it would if it were flying over areas without obstacles. Thus its ability to force the spray through the forest canopy was lost. It was for this reason that it was decided to try application by airplane the following year.

It is well known that bark beetles and ambrosia beetles often attack in large numbers at the same time, virtually as "swarms," and treatments were timed to determine whether sufficient numbers of these beetles could be killed to lower the expected level of attack. The sprays used were as follows:

1. DDT (1951): 2 pounds in 2 gallons per acre; three sprays on each DDT plot, applied on the following dates: May 23, June 25, July 25.
2. Lindane (1951): 0.2 pound in 2 gallons per acre; three sprays, applied on the following dates: May 23, June 25 and July 25.
3. DDT (1952): 4 pounds in 4 gallons per acre; two sprays on each DDT plot, applied on the following dates: June 3–4 and June 24–25.
4. BHC (1952): 0.4 pound of gamma isomer in 4 gallons per acre, two sprays, applied on the following dates: June 3–4 and June 24–25.

ROUTINE OF OBSERVATIONS IN 1951.—Examination of the bark of study trees for bark beetle entry was made at intervals during the season, and at the end of the summer (between Aug. 14 and Aug. 30) samples of bark were removed from the study trees for more detailed examination and study of the progress of the various insects.

Infestations differed enough from the crown to the butt to warrant sampling at both these points. The condition of the center section was usually either similar to the butt, or reflected conditions of both butt and crown; therefore, in order to have more time for crown and butt sampling, the center sections were generally omitted from the routine of bark removal.

A 1-foot width of bark in a ring around the stem was considered a sample. The crown sample was taken at a 6-inch diameter, the butt sample at any convenient point along the first 20 feet from the root collar. Bark was actually removed only from down trees, since it was desired to save leaners and standing trees for further study.

The data taken on each study tree included the location of the sample, the area of the bark removed in square feet, the number of galleries of each insect

Table 1.—Summary of bark sample examinations in 1951.

Treatments: spray plots #1, #2A and #3 received 2 lbs. DDT in 2 gal. oil per acre
spray plot #2B received 0.2 lb. lindane in 2 gal. oil per acre
Check plots were untreated.

Plot No.	Sample	Total Bark Area Sampled (Sq. Ft.)	Average Number Galleries per Sq. Ft. of Bark of:									
			Cerambycids	Ambrosia beetles	P. ruf.	D. picea	D. piceaperda	Ips pini	C. atomus	D. near betulae	Curculionids	Buprestids
Spray 1	Butt	128.8	0.8	0.6	1.04	0.1	0.01	0.0	0.2	0.01	0.1	0.02
	Crown	52.0	0.9	0.4	9.1	0.4	0.1	0.0	0.0	0.0	0.6	0.02
Check 1	Butt	28.2	0.9	0.3	0.8	0.2	0.0	0.0	0.0	0.0	0.0	0.0
	Crown	12.9	0.8	1.2	7.4	0.9	0.0	0.0	0.0	0.0	1.6	0.0
Spray 2A	Butt	15.5	0.6	0.0	1.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0
	Crown	7.4	0.8	0.3	6.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Spray 2B	Butt	23.1	0.5	0.04	1.8	0.0	0.0	0.0	0.0	0.0	0.3	0.0
	Crown	9.5	1.3	0.1	6.1	0.0	0.0	0.5	0.0	0.0	4.3	0.0
Check 2	Butt	23.8	0.8	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.04	0.0
	Crown	10.7	1.7	0.0	2.8	0.0	0.0	0.0	0.0	0.0	3.1	0.0
Spray 3	Butt	36.7	1.1	0.05	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Crown	16.2	5.1	2.8	13.6	0.9	0.0	0.3	0.0	0.0	0.0	0.0
Check 3	Butt	29.3	1.1	0.0	2.6	0.2	0.0	0.0	0.0	0.0	0.2	0.2
	Crown	12.8	4.9	1.2	20.2	0.5	0.0	0.5	0.0	0.0	0.0	0.0

species found, and their stages of development. The approximate length (height) of the tree, its DBH, its position, and whether it was living, dying or dead, were also recorded, together with comments on moisture, degrees of necrosis beneath bark and any special or unusual features characteristic of any given tree.

ROUTINE OF OBSERVATIONS IN 1952.—The same routine of observations followed in 1951 was continued in 1952. In June, 1952, of course, the bark beetles and other beetles in the blowdown plots had had an entire year in which to pursue their activities, and many trees which in 1951 were unsuitable for attack had reached a suitable stage in 1952.

The data taken and biological observations made afford the basis of evaluating the sprays. The average number of galleries per square foot of bark was used as the unit of measure and results are presented in tables 1 and 2:

Explanation of Table Headings

Ambrosia beetles

T. biv. = *Trypodendron bivittatum* Kirby
G. mat. = *Gnathotrichus materiarius* Fitch

Bark beetles

P. ruf. = *Polygraphus rufipennis* Kirby
D. piceae = *Dryocoetes piceae* Hopk.
D. near betulae = *Dryocoetes* Eichh. near *betulae*
D. piceaperda = *Dendroctonus piceaperda* Hopk.
C. atomus = *Crypturgus atomus* Lec.

Cerambycids, buprestids and curculionids were larval determinations.

It may be seen from the data presented that no reduction in beetle populations was obtained by the air sprays in either year.

Hand Spray Experiments.—It was desirable to know how hand spraying of windthrown trees would compare with aerial spraying in the same general area. Therefore, in the spring of 1951, 10 windthrown red spruce were selected for the experiment near one of the check plots. Eight large sections of boles of these trees were chosen for treatment. The sections were of merchantable size from the standpoint of salvage.

The sprays were applied with a 3-gallon air compressor sprayer using 35 lbs. pressure. A half gallon of spray was applied to the run-off point completely around the bole. The formulations used were prepared from a 20 per cent emulsifiable lindane and diluted with water to the following:

Treatment	*Per Cent lindane*
A	0.125
B	0.25
C	0.5
D	1.0

The sprays were applied in two series; series #1 on May 30 and series #2 on June 12. Each of the eight selected bole sections received an application of spray.

Examination of windthrown spruce in May showed no beetle attack but by

Table 2.—Summary of bark sample examinations in 1952.

Treatments: spray plots #1 and #3 received 4 lbs. DDT in 4 gal. oil per acre
spray plot #2 received 0.4 lb. BHC gamma isomer in 4 gal. oil per acre
(Both sprays were dispersed at rate of 2 gal. per acre applied twice)
Check plots were untreated.

Plot No.	Sample	Total Bark Area Sampled (Sq. Ft.)	Average Number Galleries per Sq. Foot of Bark of:									
			Cerambycids	Ambrosia Beetles T. biv.	Ambrosia Beetles G. mat.	P. ruf.	D. picea	D. piceaperda	C. atomus	D. Near betulae	Curculionids	Buprestids
Spray 1	Butt	146.8	1.6	1.6	0.3	10.9	1.8	0.2	0.1	[1]		
	Crown	55.8	0.9	0.2	0.0	22.0	0.3	[1]	0.3	0.0	0.3	0.0
Check 1	Butt	49.0	1.2	2.9	1.0	10.6	0.7	0.2	0.0	0.0	[1]	0.0
	Crown	17.2	0.9	1.3	0.0	25.5	0.1	0.1	0.0	0.0	1.2	0.0
Spray 2	Butt	51.1	1.4	1.9	0.5	5.9	1.2	0.0	0.1	0.0	0.3	0.0
	Crown	22.8	0.7	0.4	0.1	14.5	1.2	0.0	0.2	0.0	0.7	0.0
Check 2	Butt	26.8	2.8	1.4	0.0	2.9	0.1	0.0	0.0	0.0	2.5	0.0
	Crown	12.6	0.5	0.0	0.0	21.0	0.4	0.0	0.0	0.0	5.7	0.0
Spray 3	Butt	47.0	4.0	5.5	0.9	0.6	9.1	0.0	[1]	0.0	0.0	0.0
	Crown	18.3	2.3	1.4	0.0	15.9	0.4	0.0	0.0	0.0	0.2	0.5
Check 3	Butt	36.6	0.4	1.5	0.2	9.9	9.0	0.0	0.0	0.0	0.0	0.0
	Crown	14.1	0.9	0.0	0.0	14.1	7.4	0.0	0.0	0.0	0.0	0.0

[1] Indicates less than 0.1 gallery per sq. ft.

June 12 there were signs of some ambrosia beetle, *Trypodendron bivittatum*, and bark beetle, *Polygraphus rufipennis*, activity in the area.

In August 1952, bark samples were removed for the treated sections and data recorded in the same manner as in the study plots. Data collected are presented in the following table.

Although it was not certain whether or not the bole sections were entirely free from attack when treated, the results obtained indicate that protection was obtained according to the dosage of lindane applied and the highest dosage gave the best protection.

GENERAL SURVEY.—In 1952, toward the end of the summer, inspection visits were made to blowdown areas other than the Stratford region, to determine to what extent beetle invasions in these areas were following the same patterns. The survey was made from July 21 to August 1, and as many different types of blow-

Table 3.—Summary of bark sample examinations on hand sprayed trees in 1951.

Tree Section	Bark Area Sampled (Sq. Ft.)	Average Gallaries per Sq. Ft. of					
		Ambrosia beetles T. biv.	Ambrosia beetles G. mat.	Bark beetles P. ruf.	Bark beetles D. piceae	Cerambycids	Curculionids
A-1[1]	3.4	4.4	0.6	0	0		0
B-1	4.4	3.2	0	0	0	0	0
C-1	4.5	3.1	0	0	0	0	0
D-1	3.7	1.1	0	0	0	0	0
A-2	2.2	1.8	0	0	0	0	0
B-2	2.5	0.8	0	0	0	0	0
C-2	3.7	0	0	0	0	0	0
D-2	3.3	0	0	0	0	0	0
		Untreated checks					
E-1	2.8	13.2	0	21.2	1.4	5.0	0
F-1	1.9	8.9	0	2.1	0	0.5	1.0
G-1	1.8	6.7	1.7	4.4	0	0	0
Ck #1	4.1	7.1	1.0	2.9	0.2	3.4	0
Ck #2	5.4	7.6	0	1.0	3.5	2.6	0

[1] Letters A, B, C & D indicate spray treatments as follows:

Treatment	*Per Cent lindane*
A	0.125
B	0.25
C	0.5
D	1.0

down as possible were visited, including those where salvage had been completed. In all, 30 areas or sites were visited, and notes were taken on the species of beetles present and their relative abundance. These are presented here, together with notes taken on the study plots at Stratford in 1951 and 1952.

Observations.—Following is a list of beetles found in or on windthrown trees, with host and locality records, and miscellaneous notes on certain species. Under each grouping they are listed in order of relative abundance as observed.

Ambrosia beetles—*Trypodendron bivittatum* Kby. Stratford, Cranberry Lake, North Osceola, Harrisville, Speculator, Raquette Lake, Long Lake, Lake Placid and Tahawus. In red spruce, hemlock, balsam fir, eastern larch and white pine. By far the most abundant of all the ambrosia beetles in softwood; heaviest attacks on red spruce and hemlock. Common in both 1951 and 1952 in Stratford.

Gnathotrichus materiarius Fitch. Stratford, Woodgate, Speculator, Indian Lake, Raquette Lake, Long Lake, Lake Placid and North Osceola. In red spruce, white pine, Scotch pine and balsam fir. Common in both 1951 and 1952 in Stratford. Very numerous in some areas.

Xyloterinus politus Say. Stratford, Speculator, Fort Ann, Axton, North Osceola, Harrisville and Wilmurt. In beech, yellow birch, hard maple, soft maple, black cherry and red spruce (rare in red spruce). The most abundant ambrosia beetle in the hardwoods.

Monarthrum mali Fitch. Stratford, Fort Ann, North Osceola and Wilmurt. In soft maple, beech, white ash and black cherry; more rarely in red spruce and hemlock. As with *Xyloterinus politus*, it has apparently increased in 1952 as compared with 1951, a fact true in general of hardwood species of other types of beetles as well. Near Fort Ann, a typical hardwood area, ash, especially, was very severely damaged, with galleries penetrating 4 to 5 inches. Next to ash, cherry was most heavily attacked, hard maple the least.

Anisandrus pyri Peck. North Osceola and Stratford. In beech. Not common.

Bark beetles—*Polygraphus rufipennis* Kby. (The "4-eyed" spruce bark beetle.) Stratford, Tupper Lake, Tahawus, Cranberry Lake, North Osceola, Harrisville, Woodgate, Axton, Speculator, Indian Lake, Raquette Lake, Long Lake, Graphite, Saranac Lake and Lake Placid. In red spruce, white pine, balsam fir and eastern larch. By far the most common bark beetle in red spruce, being found in all of the typical blowdown areas, and very abundant in 13 of them. Often found attacking weakened trees, especially leaners.

Dryocoetes piceae Hopk. Stratford, Lake Placid, Cranberry Lake, North Osceola, Harrisville, Speculator, Raquette Lake, Long Lake, Graphite, Axton and Saranac Lake. In red spruce. Often extremely numerous, sometimes more numerous than *Polygraphus rufipennis*. Whereas *P. rufipennis* was found in flight during most of the season, *D. piceae* had a limited flight period.

Ips pini Say. Speculator, Lake Placid, Cranberry Lake and Woodgate. In white pine and Scotch pine. Also at Stratford in red spruce.

Orthotomicus caelatus Eichh. Stratford, Speculator, Indian Lake, Long Lake, Lake Placid, Cranberry Lake and Woodgate. In red spruce, white pine, balsam fir and Scotch pine.

Crypturgus atomus Lec. Stratford, Long Lake Saranac Lake, Cranberry Lake, Woodgate and Axton. In red spruce.

Pityoktienes sparsus Lec., (the "balsam fir beetle"). Stratford, Indian Lake, Cascade Lake and Boreas River. In balsam fir.

Pityogenes hopkinsi Sw. Long Lake, Graphite, Lake Placid. Found attacking live, dying and dead balsam fir.

Dendroctonus piceaperda Hopk., (the eastern spruce beetle). Stratford, Raquette Lake, Middle Saranac Lake, Lake Placid. In red spruce. It was abundant only near Middle Saranac Lake, where it was attacking live leaners.

The fact that much potential breeding material was already pre-empted by *Polygraphus rufipennis* may have inhibited attack by *Dendroctonus piceaperda*. However, there continues to be much favorable breeding material, and unfavorable conditions at any time can bring additional weakened trees, or trees in unfavorable condition to the susceptible stage. Since four of the six *Dendroctonus* sites were located in the Saranac-Placid area, this region may be a potential danger zone and will be subjected to more intensive scouting.

Dendroctonus simplex Lec., (the eastern larch beetle). Saranac Lake and Stratford. In red spruce.

Ips grandicollis Eichh. Speculator and Woodgate. In Scotch pine and white pine.

Scolytus piceae Sw. Tahawus. On bark of balsam fir.

Hylurgops pinifex Fitch. Woodgate. In Scotch pine.

Dryocoetes betulae Hopk. Stratford. In yellow birch.

Dryocoetes near *betulae*. Stratford. In red spruce.

Phthorophloeus liminaris (Harr.). Wilmurt. In the bark of black cherry.

Wood borers—1. Buprestidae. *Melanophila fulvoguttata* Harr., (the hemlock borer). Stratford, Long Lake and Axton. In hemlock. Abundant and may be a threat to weakened trees.

Chrysobothris scabripennis C. & G. Stratford. From spruce and hemlock.

Buprestis maculaventris Say. Stratford and Graphite. From hemlock and balsam fir.

Dicerca divaricata Say. Graphite. From balsam fir.

2. Cerambycidae. *Monochamus scutellatus* Say. Stratford and probably present in all of the other areas where there was down spruce and pine. In red spruce, white pine and balsam fir. Greatly increased numbers in 1952.

Monochamus notatus Drury. Stratford. In red spruce.

Tetropium cinnamopterum Kby. Stratford. In red spruce.

Anoplodera mutabilis Newm. Stratford. Off red spruce.

Anoplodera minnesotana Csy. Stratford. Off black cherry and red spruce.

Anoplodera vittata Oliv. Stratford. Off red spruce.

Anoplodera biforis Newm. Woodgate. Off white pine.

Anthophylax viridis Lec. Stratford. Off balsam fir.

Asemum atrum Eichh. Stratford. Off red spruce.

Callidium violaceum L. Stratford. In red spruce.

Clytus ruricola Oliv. Stratford. Off hemlock, red spruce and hard maple.

Evodinus monticola Rand. Off red spruce.
Leptura lineola Say. Stratford. From flowers. Has been reported breeding in birch and ironwood.
Leptura subhamata Rand. Stratford. In red spruce.
Typocerus velutinus Oliv. Stratford. From hardhack flowers. Has been reported from numerous hard and softwood hosts.
Xylotrechus fuscus Kby. Stratford. Off red spruce.
Stenocorus lineatus Oliv. Woodgate. In white pine.
Acanthocinus pusillus Kby. Stratford. Off red spruce.
Bark weevils—Curculionidae. *Eupsalis minuta* Drury. Stratford. Off red spruce.
Pissodes dubius Rand. Stratford, Lake Placid and Indian Lake. In red spruce and balsam fir.
Pissodes fiskei Hopk. Stratford. In red spruce.
Pissodes rotundatus Lec. Stratford. In red spruce.

DISCUSSION AND CONCLUSIONS.—It was apparent from the survey of blowdown areas that the study plots at Stratford were, in general, representative of blowdown conditions throughout the Adirondacks. It was also evident that the beetle populations have greatly increased between 1951 and 1952 due to the vast amount of available breeding material. Where blowdown material is still green because of sufficient moisture to maintain it, or because the trees originally were not badly damaged, the beetles will continue to attack as the material becomes suitable for the different species to breed in it. This potentially suitable material is small in amount compared to the total amount originally blown down. It is in this residue that many of the beetles of economic importance will continue to breed. However, there are good indications that in most places, secondary species dominate and will attack this material, thus providing heavy competition for the primary species.

Most of the softwood blowdown material died the first year, that is in 1951 and became generally infested that year. This material is no longer suitable for ambrosia beetle attack or further attack by wood borers ordinarily attacking green timber. Much damage has already been done and will continue where borer larvae are still present in the wood.

Most of the hardwood blowdown material did not die until the summer of 1952 and much of it is still green so that this material remains subject to attack, particularly by ambrosia beetles. Attack has already been noted and will continue as long as the material remains suitable for breeding the ambrosia fungus. A few cerambycid and buprestid larvae have been found under the bark of hardwoods but very little damage to wood has yet been noted.

Although aircraft spraying to control beetle populations attacking blowdown was not successful with the equipment and materials used, hand spraying of logs or boles of trees does promise protection from beetle attack, especially when complete coverage is applied. Protection for two seasons is apparently sufficient to prevent attack by ambrosia beetles and wood borers attacking green timber.

The information gained in the two seasons of work in the blowdown study plots and in the general survey as outlined above is now being used as a background for further work which has as one of its specific objectives an analysis of the remaining blowdown material, both hardwoods and softwood, in terms of the current and future status of its salvability.

Dr. F. C. Bishopp was elected president of the American Mosquito Control Association at the annual meeting in Daytona Beach, Florida, in April. The Association will hold its 1954 meeting jointly with the New Jersey Mosquito Extermination Association in Atlantic City, March 9 to 12, 1954.

Control of Native Rodent Fleas with DDT Applied to Simulate Aerial Dusting

VIRGIL I. MILES and MAXWELL J. WILCOMB, JR.[1]

With the discovery of several foci of campestral or "sylvatic" plague in West Texas in 1947–1949 (1), the need was apparent for a practical method of controlling fleas over wide areas of native rodent habitat in the event of plague spread to centers of human populations. Considering the possibility of effective flea control over such areas by widespread aerial applications of DDT dust, experiments were conducted in August 1948 on control of fleas among native rodents by DDT applied in a manner simulating airplane dusting. The objectives were to determine (1) the reduction, if any, in numbers of fleas on native rodents by this method of DDT application, (2) the amount of DDT required for effective reduction of fleas under these conditions, (2) the effect of DDT on native rodents and other wildlife when applied in dosages required for effective flea control, and (4) the duration of DDT effectiveness as influenced by weathering, rodent movements and other factors. The experiments were performed with limited facilities to obtain general information which may be useful in planning more detailed and extensive experiments.

THE EXPERIMENTAL AREA.—On a ranch about 10 miles northeast of Seminole in Gaines County, Texas, three plots, one each of 3, 8 and 15 acres, were selected for the experimental dusting. The area chosen contained intermittent patches of mesquite bushes (*Prosopis chilensis*) 3 to 6 feet tall, favorite habitat in the region for the packrat, *Neotoma micropus*. In selecting plots for dusting, an attempt was made to discover areas with high packrat populations but with comparatively well-defined borders of shinnery oak (*Quercus breviloba*), short grass, or other cover not inhabited by packrats. This was for the purpose of reducing interchange of packrats between dusted and undusted areas. The areas selected were fairly well isolated by the absence or relative paucity of packrat dens beyond the boundaries on at least three sides of the dusted areas. Two additional areas with similar mesquite cover were chosen as control plots, one being about one-half mile from the 15-acre plot, the other being separated from the 3-acre and 8-acre plots by a draw approximately 100 yards wide with scant cover.

METHODS.—Acreages of the dusted plots were computed from measurements taken by use of a 10-yard length of rope and a hand compass. The plots were dusted as shown in the following table:

PLOT	PER CENT DDT DUST USED	TOTAL LBS. DUST APPLIED	LB. PURE DDT/ACRE	DATE APPLIED
15-acre	10	150	1	Aug. 10
3-acre	10	90	3	Aug. 11
8-acre	25	160	5	Aug. 10 & 11

The dust was applied with hand-operated "Savage" rotary dusters by four men walking across the plots. Paths of these men were about 5 to 10 yards apart, depending upon the extent of drift of the dust caused by variable breezes. The lee sides of the plots were dusted during periods of calm in order to minimize the amount of drift beyond the measured boundaries. The areas were repeatedly covered until the established dosages of DDT had been applied. The distribution was general and even, with no particular attention being given to packrat dens or areas of exceptionally high rodent population, so as to simulate dusting by airplane as closely as possible.

In sampling the flea populations of the areas, special attention was given to taking packrats because of their comparatively large numbers and high flea populations. All rodents were taken alive in number "O" Victor steel traps, and when removed from the traps were immediately placed in flea-tight cloth bags. After a 12- to 15-minute exposure to hydrocyanic acid gas, the animals and everted bags were carefully examined for removal of all fleas. The fleas were sent to the Bureau of Laboratories, Texas State Department of Health, for identification.

Dusted and control areas were trapped

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during the week before dusting and during the first, second, and fourth weeks after dusting, as shown in tables 1 and 2. It was believed that analysis of results should be based on reduction of non-sticktight fleas because the sticktight species, *Echidnophaga gallinacea*, particularly the female, does not normally leave its host and would not be likely to come in contact with the DDT as here applied, *i.e.*, not directed into the burrows. The numbers of non-sticktight fleas and a comparison of their abundance with that of sticktight fleas were therefore computed from the flea recoveries. The non-sticktight species, *Orchopeas sexdentatus*, comprised 94 per cent of the total number of fleas taken throughout the experiment and is not considered separately in the tabulations.

RESULTS.—The results of the experiment are summarized in tables 1 and 2. The average numbers of fleas per packrat in each sampling are shown graphically in figures 1 and 2. A reduction in populations of non-sticktight fleas is indicated 1 week after dusting with 5 pounds of pure DDT per acre. The results suggest that 1 and 3 pounds of pure DDT per acre also caused some reduction of these fleas, based on the ratios of sticktight to other fleas in the dusted areas as compared to these in the control areas one week after dusting. The samples taken the second and fourth weeks after dusting show no discernible differences in the ratios of sticktight to non-sticktight fleas in dusted and control areas. There was no significant change in sticktight flea populations before and after dusting.

There is no evidence that the DDT dust affected the native rodents. While the populations of packrats obviously declined with each successive trapping in dusted areas, particularly in the 3-acre and 8-acre plots, this was believed due to reduction by trapping. The control areas did not show this decline, but these areas were larger (25 to 30 acres), were not isolated as were the dusted plots, and therefore had a larger total population.

It was noted that there was a marked reduction in the insects commonly prevalent, particularly the ants and grasshoppers, in all dusted areas until the second week after dusting. These insect populations apparently returned to normal within 4 weeks after dusting. No birds, mammals, or reptiles were found dead or affected at any time during the experiment.

The records show that the effectiveness of the DDT dust was lost by the second week after dusting. There were no rains, heavy dust storms or other adverse weather conditions during the experiment to affect the duration of DDT effectiveness other than exposure to normal sunshine and the moderate winds prevalent in the area.

DISCUSSION.—On the basis of trapping success in the areas one week before dusting, it was believed that a minimum of 15 packrats could be taken from each

Table 1.—Fleas from live-trapped packrats, *Neotoma micropus*, before and after aerial DDT dusting, Gaines County, Texas.

	Lbs. Pure DDT/Acre	Date Trapped August 1948	Pack-rats Trapped	Average Fleas per Rat		Ratio Stick-tight to Others
				Not Stick-tight	Stick-tight	
Before dusting (15 acres)		3, 4	20	1.6	12	7.9:1
First wk. after dusting	1	17, 18	16	1.0	14	14.3:1
Second wk. after dusting		24, 25, 26	14	2.6	19	7.3:1
Fourth wk. after dusting September		8, 9	17	4.3	22	5.2:1
Before dusting (3 acres)		3	12	1.5	16	10.4:1
First wk. after dusting	3	20, 21	11	0.8	11	13.7:1
Second wk. after dusting		27, 28	6	6.7	12	1.8:1
Fourth wk. after dusting September		9, 10	2	20	60	2.5:1
Before dusting (8 acres)		6, 7	18	14	37	2.7:1
First wk. after dusting	5	17, 18, 19	11	0.3	12	44.0:1
Second wk. after dusting		24, 25, 26	5	6.2	35	5.6:1
Fourth wk. after dusting September		8, 9, 10	4	5.3	30	5.8:1

Table 2.—Fleas from live-trapped packrats, in control areas, Gaines County, Texas.

Date Trapped August 1948	Number of Pack-rats Trapped	Average Fleas per Rat		Ratio Sticktight to Others
		Non-Stick-tight	Stick-tight	
Control Area Near 3 and 8-Acre Areas				
Pre-dusting period 4, 5	13	1.6	18	11.1:1
First week 20, 21	10	5.1	22	4.2:1
Second week 27, 28	11	4.9	25	5.1:1
Fourth week September 10	15	1.0	5.1	5.1:1
Control Area Near 15-Acre Area				
Pre-dusting period 3, 4, 5, 6, 7	25	2.7	15	5.4:1
First week 17, 18, 19	12	2.6	12	4.6:1
Second week 27, 28	17	4.0	8	1.9:1
Fourth week September 8, 9, 10	22	4.2	12	2.9:1

area in successive re-trappings the first, second and fourth weeks after dusting. It became increasingly difficult, however, to secure adequate samples with each successive trapping, especially in the 3-acre and 8-acre plots. The reduction of rats by intensive trapping may have caused the fleas to concentrate on the fewer hosts remaining, as observed by Cole & Koepke (2). The later trappings resulted in captures of increased proportions of young packrats; some probably came from burrows of trapped adult rats. It was also noted that the later trappings were somewhat more successful near the margins, particularly of the smaller plots, suggesting that some of the rats examined probably entered the dusted plots from outside areas. These factors may partially account for the apparent rapid restoration of flea populations indicated during the second and fourth weeks after dusting. The return of other insects, however, about two weeks after dusting, substantiates the flea record in showing that the effectiveness of the DDT was rapidly lost. Its exposure to sunshine, and the mixing and covering of the DDT with the dusty soil, even by gentle breezes, were probably the principal factors contributing to its degeneration.

While the results of the pilot project here described are not sufficiently detailed for conclusions regarding the feasibility of applying DDT by air for control of native rodent fleas, the possibility of emergency, temporary reduction of these fleas is indicated, with due consideration for climate, soil and vegetation types, host species, and other factors affecting this method of control.

SUMMARY.—Three of five ranchland plots infested with packrats, *Neotoma micropus*, and fleas were dusted with pyrophyllite-DDT mixtures at dosages of 1, 3 and 5 pounds of pure DDT per acre, respectively. The dust was applied by hand-operated rotary dusters in a manner simulating airplane dusting. Flea recoveries from packrats trapped alive from all plots one week before and 1, 2 and 4 weeks after dusting showed a reduction of non-sticktight fleas was effected by 5 pounds DDT per acre, and a suggestion of some reduction of these fleas by 1 and 3 pounds DDT per acre 1 week after dusting. The DDT effectiveness apparently was lost within 2 weeks. Sticktight fleas, *Echidnophaga gallinacea*, were not significantly reduced by this method of DDT application. Other insects prevalent in the area were markedly reduced, but no effect of the DDT was noted on other forms of wildlife. The general information acquired from the experiment, which was executed with limited facilities, may serve as a guide in designing more extensive tests on flea control by aerial DDT dusting.

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Dispersal Habits of *Phormia regina* in Charleston, West Virginia

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The tendency of certain blowflies, such as *Phormia regina* and *Callitroga macellaria*, to disperse more rapidly and to greater distances than the house fly, *Musca domestica*, has been demonstrated by several workers (Bishopp & Laake, 1921, Lindquist *et al.* 1951, Yates *et al.* 1952). In the studies by Yates *et al.* in rural Oregon, the 87 per cent recovery levels for tagged *Musca domestica* and *Phormia regina* released at the same point were obtained within the ranges of 1.0 and 3.5 miles, respectively, for the two species. At Savannah, Georgia, Quarterman *et al.*[1] established that *Callitroga macellaria* and *M. domestica* ranged freely over the community up to 4 miles or more, the former species exhibiting the greater range of movement.

On the postulate that blowflies, because of free access to pathogenic sources and human foods may serve as a vehicle of disease transmission, it follows that the extended dispersal range of these flies increases immeasurably their potential health hazard. To obtain further insight into the dispersal habits of *Phormia regina* in urban areas, tests were conducted in Charleston, West Virginia, during 1951 and 1952. Principal aim of the study described was to determine the movement of *P. regina* between two areas of contrasting socio-sanitational levels, located 6.5 miles apart. Both of the areas in question were under intensive epidemiologic surveillance as a routine part of the Poliomyelitis Investigations-Fly Control Program now in operation at Charleston.

Description of Study Area.—The study area consisted of two cities, Charleston (72,818) and South Charleston (16,627), West Virginia. Principally noted for their industrial output, these adjoining communities lie on the banks of the Kanawha and the Elk Rivers (Fig. 1) in the Kanawha Valley. The topography adjacent to the rivers is flat, but beyond the valley floor, the terrain is hilly and densely wooded, rising to elevations 400 to 500 feet above the valley floor. As a result, the two cities extend over an elongate expanse 10.5 miles long and 1.5 to 3.0 miles in width. The major industries and business establishments are located adjacent to the rivers while the residential developments occur both in the valley and upon the surrounding ridges. The topography and general characteristics of Charleston thus offer conditions different from those encountered in previous urban dispersion studies by other workers.

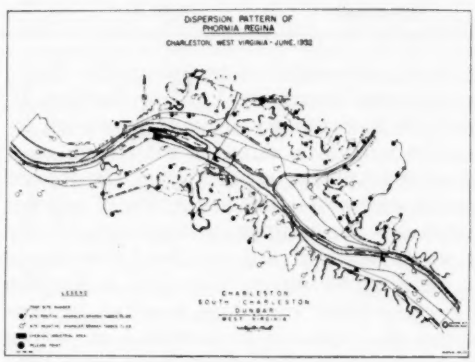

Fig. 1.—Dispersal pattern of *Phormia regina* released at Chandler Branch.

Methods.—Abnormally hot weather caused an excessive mortality in the 400,000 *Phormia regina* reared for the study. As a result, recourse was made to procurement of wild flies at extraurban abattoirs. Over a 2-day period, an estimated 50,000 flies were collected at four sites. All specimens were transferred to a single abattoir to facilitate the feeding of the radioactive milk to the flies.

The radioactive solution was prepared by adding 1 millicurie of P-32 as phosphoric acid to 1 liter of milk (evaporated milk, honey, and water). Feeding of the flies was accomplished by the gravitational flow technique (Schoof *et al.* 1952), the specimens having access to the isotope solution for a period of 20 to 24 hours. Prior to the introduction of the radioactive milk, the flies were fed a water-honey solution.

[1] Quarterman, K. D., Mathis, W. and J. W. Kilpatrick. (Unpublished manuscript.) "Fly dispersion in urban areas of southern United States." Communicable Disease Center, Savannah, Georgia.

Shortly after nightfall on June 20,[2] approximately 12,000 specimens (60 to 70 per cent *Phormia regina*) were transported to each of two release sites (Chandler Branch and Kanawha City). At the liberation sites, each cage of specimens was placed in a card-board container and the flies dusted with a distinguishing red or green dye.[3] Immediately after being dusted, the specimens were released. Examination of fly samples from each site revealed 100 per cent positive marking with the P-32 and the particular dye employed.

A total of 87 attached-bait pan traps (Schoof 1952) was distributed over the study area to retrieve marked flies. Principal emphasis was placed upon locating trap stations in the urbanized area, no attempt being made to sample the extraurban zones beyond the two release points. Despite the selection of trap stations on the basis of the general fly attractivity of the immediate area, many of the sites, of necessity, were in environments without evident fly attrahents. Because of the nature of the topography and the irregular limits of the two communities involved, the greater percentage of traps in relation to Chandler Branch fell in the range of 0.7 to 4.0 miles, those in relation to Kanawha City, 4.0 to 9.0 miles.

All traps operated continuously for a 10-day period following the release on June 20. The bait consisted of fish heads, each trap receiving fresh bait on alternate days. The initial collections began at noon of the day following release; after which flies were removed from the traps on a daily schedule except for one combined sample for June 28 and 29.

To detect the radioactive flies, the collections were processed by a laboratory or field monitor equipped with a thin-walled Geiger tube. After detection, each radioactive specimen was treated with acetone to expose the dye characteristic of the point of release.

The release points employed (approximately 6.5 miles apart) represent different socio-economic groups. Chandler Branch is characterized by crowded living conditions, substandard dwellings, privies, and a generalized low level of environmental sanitation. This zone, located on the northwest edge of the city, centers in a small valley on the edge of a polluted creek. In contrast, Kanawha City lies on a flat plain adjacent to the Kanawha River at the southeast tip of Charleston. Sanitary and living conditions in Kanawha City are representative of the highest standards existent in the Charleston-South Charleston area.

RESULTS.—Collections for the first post-release day yielded only one marked fly, the low recovery rate presumably being the result of rain on the morning of the day after release. At the end of 48 hours, 16 tagged flies were recaptured in the range of 0.7 to 3.1 miles. On the third post-release day, maximum recovery oc-

[2] Liberation occurred on Friday to provide a 2-day period free of space spray treatments in the cities.

[3] Dyes from the Pylan Products, New York. (Although these dyes were used in this particular experiment, it is assumed that selected dyes from other manufacturers would be equally effective.)

Table 1.—Recovery pattern of radioactive *Phormia regina* liberated from two release points.

CHANDLER BRANCH				KANAWHA CITY		
Number Trap Sites	Number Trap Sites Positive	Number *P. regina*	Miles	Number *P. regina*	Number Trap Sites Positive	Number Trap Sites
5	5	15	0.7– 0.9	0	0	2
17	7	11	1.0– 1.9	6	3	5
21	8	16	2.0– 2.9	4	3	4
21	13	21	3.0– 3.9	4	4	10
13	5	7	4.0– 4.9	4	3	17
10	0	0	5.0– 5.9	0	0	12
			6.0– 6.9	1	1	11
			7.0– 7.9	2	2	9
			8.0– 8.9	1	1	9
			9.0– 9.9	3	3	6
			10.0–10.9	1	1	2
87	38	70		26	21	87

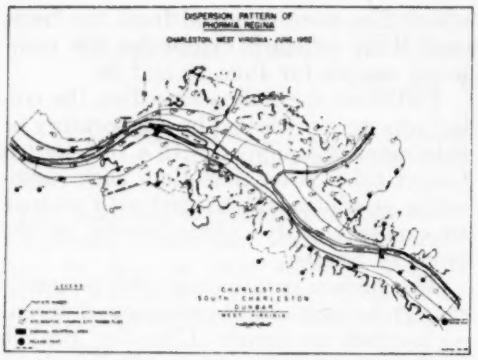

FIG. 2.—Dispersal pattern of *Phormia regina* released at Kanawha City.

curred with 36 radioactive specimens being recaptured between 0.7 and 9.3 miles. Maximum dispersion was 10.3 miles on the fourth post-release day.

Of the 87 sampling points, 44.8 per cent were positive for flies from Chandler Branch, 22.9 per cent for flies from Kanawha City. Ten trap stations yielded flies from both release points. The number of flies recovered per individual site was relatively low (maximum yield—7 flies), an indication that the stations selected did not differ materially in their fly-attractive value.

The collection data are summarized in table 1. Principal recovery range from both liberation sites was within 5 miles, 90 per cent of the Chandler Branch recoveries being between 0.7 and 3.9 miles. A total of 96 radioactive and colored *Phormia regina* was collected, this number representing a recovery of less than 1.0 per cent of the total number released. Only 10 of the recaptured specimens were males, all of which were recovered within the range of 0.7 to 4.5 miles. The capture of undyed radioactive flies, particularly at the feeding station, indicates the necessity of marking all radioactive specimens with differentiating dyes when two or more release sites are employed. Non-colored radioactive flies could represent inadequately-dusted specimens, flies which escaped from the holding cages before being dusted, or uncaged flies feeding upon radioactive material within the cage, thus rendering any attempt to include them in the findings a potent source or error.

As the number of tagged specimens collected per positive station was relatively uniform, the pattern of dispersion can be traced on a qualitative basis. Thus, Figure 1 reveals that the flies released at Chandler Branch spread in all directions over the study area regardless of the presence of intervening ridges or water courses between the liberation site and trap stations. No marked directional movement was apparent in the dispersal, 45 per cent of sites either southeast or southwest of the Chandler Branch producing radioactive flies from that release point.

The *Phormia regina* liberated at Kanawha City were taken less readily than those from Chandler Branch, only one specimen entering the trap during the first two post-release days. The lesser number of specimens retrieved from the Kanawha City liberation can be ascribed to a trapping pattern which, based on the configuration of the community limits, established the majority of the traps in the range of 4.0 to 9.0 miles. Within the study area, the positive stations indicate two paths of movements. One extends in a northerly direction across the Kanawha River through sites 2, 6, 10, 11, 17, to stations 18 and 19, 3.5 to 4.0 miles distant. The second path of dispersion runs northwest through the foothills (sites 12, 13, 20, 21, 48, 62, 64, 65, 67, 76, 77, 78) up to 10 miles.

Consideration of negative trap sites reveal two enigmas. The first concerns the absence of any marked *Phormia* in the 11 traps servicing a compact area centered near the junction of the Elk and Kanawha Rivers. This expanse is composed primarily of commercial establishments and lies 4.0 to 5.0 miles from Kanawha City, and 1.5 to 2.5 miles from Chandler Branch. Possible explanation for the absence of positive sites therein is found in trap volume data which indicate the average quantity of flies per collection in this area to be 25.7 cubic centimeters as compared with 55.2 cubic centimeters per collection for the 47 positive trap sites. The average volume for the remaining negative sites in the city was 50.5 cubic centimeters per collection. Based on these volumetric analyses, the area in question appears to be less attractive to blowflies than does the remainder of the city.

The second oddity apparent from the negative trap collections is the almost complete failure of tagged flies from Kanawha City to enter traps located in

Charleston, north of the Kanawha River (Figure 2). The movement of *Phormia regina* south of the Kanawha River to sites in the range of 6 to 10 miles indicates that specimens were capable of penetrating to distances characteristic of the trap locations north of the river. Likewise the fact that blowflies from Chandler Branch exhibited no reluctance in crossing the river establishes that the watercourse in itself did not constitute a formidable barrier to movement. The occurrence of the majority of the positive traps in hilly terrain suggests that temperature gradients may have been an important factor influencing the dispersion pattern. During the test period, abnormally high temperatures[4] prevailed with readings in the urban zones of the valley floor being 5 to 9 degrees higher than those of the adjoining hilly and wooded areas. Since *P. regina* normally is a cool weather species, the significance of this temperature differential is augmented. This same factor could also be an underlying cause of the block of negative sites in the vicinity of the Elk and Kanawha River junction.

While *Phormia regina* comprised more than 83 per cent of the tagged specimens recovered, the remaining 17 per cent (19 specimens) represented 7 species, *Callitroga macellaria*, *Lucilia illustris*, *Sarcophaga bullata*, *Fannia scalaris*, *Musca domestica*, *Phaenicia sericata*, and *Ophyra leucostoma*. The only species recaptured in number was *O. leucostoma*, which occurred at nine stations located 1.0 to 4.5 miles from the release points.

In view of the number released, the low recovery of *Musca domestica*, although disappointing, was not entirely unexpected. In a test conducted in August 1951, only 11 house flies were retrieved from a release of 27,000 *M. domestica*. Observations indicate that in the Charleston area, this species does not enter baited traps as readily as do blowflies. Analyses of total trap captures revealed that, of the 330,000 flies recovered, 41 per cent were *Phormia regina*, 24.5 per cent *Ophyra leucostoma*, 6.7 per cent *Phaenicia* spp., with *Musca domestica* at the low level of 1.0 per cent.

DISCUSSION.—The data (Figs. 1 and 2, Table 1) show that *Phormia regina*, the black blowfly, is capable of rapid dispersal up to distances of 6 to 10 miles, the movement not being deterred by the occurrence of water barriers or wooded ridges of 400 to 500 feet in elevation. Whether the flies, in reaching stations which were separated from the release point by one or more ridges actually traversed these hills, is a moot question. Such dispersion could have beeen accomplished by specimen migration following the course of the interconnected valleys. The only established fact is that such elevations do not impose a barrier to the dispersal of *Phormia regina*.

On the premise that pathogenic organisms could survive on *Phormia regina* for periods of 48 to 72 hours, the findings reveal that this species could serve as a potential vector over an area 8 to 20 miles in extent. The pattern of dispersion does not exhibit any predilection towards specific locations such as has been shown for *Musca domestica* (Schoof *et al.* 1952). Apparently *Phormia regina* roams over a wide expanse in search of food and breeding grounds. This concept agrees with the findings of studies in rural zones and in cities lacking hilly terrain (Lindquist *et al.* 1951; Yates *et al.* 1952; Quarterman *et al.* unpub.). Since Yates *et al.* (1952) reported 8.5 per cent of the 1,606 tagged *Phormia regina* recovered to lie in the range of 11 to 28 miles, definite proof is available that this species actually is capable of substantial migration to distances beyond the 10 miles reported in this paper.

Although the data from the present test do not show a reciprocal movement of *Phormia regina* between the two release points, they reveal that blowflies frequenting the substandard areas of Chandler Branch can and does disperse to the Kanawha City section. In addition, *Phormia regina* from Chandler Branch readily migrated to all parts of the metropolitan areas and into zones which were representative of highest level of sanitation. Such migration again demonstrates that the health and freedom from insect annoyance of any part of a community are subject to influence by substandard conditions which may abound in another portion of the city.

In relation to the control of *Phormia regina* in a community, the findings vindicate the recommendation that suppressive measures for this species be extended to sources of copious blowfly pro-

[4] Average maximum temperature for 10 post-release days at airport was 91° F., in business area 100° F.

duction within 3 to 4 miles of the urban limits. Establishments such as dumps, abattoirs, rendering plants and similar problem sites which generally locate on the fringes of urbanized areas, must receive attention comparable to that given to breeding points within the city.

SUMMARY.—

1. Release of approximately 8,000 dyed and radioactive *Phormia regina* from each of two release points in Charleston, West Virginia, revealed rapid dispersal up to distances of 6 to 10 miles. The majority of the marked flies were recaptured within 5 miles of the release points.

2. Fly movement from a substandard sanitational zone to all parts of the city was evident. Migration of flies was not hampered by the presence of wooded ridges 400 to 500 feet in elevation nor by watercourses.

3. Extension of control operation to prodigious blowfly production sources 3 to 4 miles beyond city boundaries is required to effect maximum reduction of *Phormia regina* within a community.

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Newer Insecticides for Japanese Beetle Control[1]

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In communities where the Japanese beetle is a serious problem, it is essential that the people have as much information as possible on the merits of the different insecticides that might be satisfactory for its control. Excessive damage from the beetle, spray residue problems, and the toxicity of the various insecticides as related to humans have stimulated the public to demand an evaluation of practically every new insecticide with respect to its efficacy for the control of the Japanese beetle.

Evaluation studies on the relative efficiency of several of the new insecticides are available. There are several others on which little or no published information is available. Prior to the advent of DDT none of the commonly used insecticides could be considered really efficient for killing beetles or fully effective for the protection of foliage and ripening fruit. DDT proved to be an outstanding insecticide in both of these respects.

Shortly after DDT had been generally accepted as an efficient insecticide for the control of the Japanese beetle, quite a series of other organic chlorinated hydrocarbons developed for insecticidal purposes, were introduced. Many of these, notably, chlordane, TDE, methoxychlor, the gamma isomer of BHC, toxaphene, Aldrin and Dieldrin were found to be highly efficient for killing beetles. The organic phosphate, parathion, was introduced during the same period. It also proved effective.

A number of papers have been published covering the effectiveness of these insecticides. Fleming & Chisholm (1944) and Langford & Cory (1945) published on the usefulness of DDT for controlling the beetle on fruit. Langford and Squires (1947) reported on benzene hexachloride and chlordane, while Fleming & Maines (1950) published on methoxychlor and TDE. Langford, Squires & Dozier (1951) published an evaluation study of all of the above insecticides, along with aldrin, dieldrin and parathion in which the relative efficiency of the insecticides both from the standpoint of dosage and residual action was reported. Siegler (1952) published a similar study in which he evaluated the residual toxicity of these

[1] Scientific Article No. A388, Contribution No. 2404 of the Maryland Agricultural Experiment Station (Department of Entomology).

Table 1.—Showing comparative effectiveness of various newer insecticides against the Japanese beetle.

FORMULATION OF INSECTICIDES	DOSAGE-ACTIVE INGREDIENTS IN LBS. PER 100 GAL.	% KNOCKDOWN AFTER FOLLOWING INTERVALS				% DEAD	CHECK
		½ Hr.	1 Hr.	2 Hrs.	4 Hrs.	24 Hrs.	% Alive
Dilan emul. 25%	0.5	100	100	—	—	100	100
2-nitro-1, 1-bis (p.-chlorophenyl) propane	0.25	98	100	—	—	100	100
+2-nitro-1, 1-bis (p.-chlorophenyl) butane	0.125	72	89	77	86	81	100
EPN W. P. 27%	1.0	2	2	2	5	26	100
Ethyl'p-nitrophenyl thiono benzene phosphanate	2.0	19	42	49	65	100	100
	3.0	49	57	73	100	100	100
Malathon emul. 50%	1.0		42	63	85	91	100
0,0-dimethyl dithiophosphate of diethyl mercaptosuccenate	2.0	36	82	93	100	100	100
	3.0	86	100	100	100	100	110
Metacide emul. 6.3%	0.125	99	100	100	100	100	100
Parathion+	0.0625	91—	98	100	100	100	100
Dimethyl homologue	0.03125	63	63	88	98	100	100
Octomethyl pyro-phosphoramide emul. 70%, and related organic phosphate, 20%	1.0	5	8	13	24	57	100
	2.0	1	9	15	30	88	100
	3.0	7	13	29	31	100	100
NPD emul. 85%	1.0	4	4	5	5	39	100
Tetra-n-pyrophyl dithienopyrophosphate	2.0	6	11	18	21	100	100
	3.0	27	36	36	61	100	100
Systox emul. 32.1%	1.0	7	15	22	98	100	100
Trialkyl thiophosphate	0.25	34	43	88	97	100	100
	0.125	1	1	2	5	80	100
DDT emul. 30%	1.0	81	86	87	87	97	100
	2.0	76	82	84	88	99	100
	3.0	93	94	99	100	100	100
Lindane emul. 20%	0.25	92	100	—	—	100	100
	0.125	96	100	—	—	100	100
	0.0625	72	89	90	100	100	100
Methoxychlor emul. 20%	1.0	—	97	—	—	100	100
	1.5	100	100	—	—	100	100
	2.0	100	100	—	—	100	100

same insecticides with the exception of TDE. He added EPN.

Since the publication of the above studies, other new organic insecticides have been developed, and are being offered for commercial use, or experimentation in the control of insects. Research at the University of Maryland indicates that some of these, notably Dilan, malathon, EPN, NPD, and metacide have promise for use in Japanese beetle control work. Two systemics; namely, systox and octomethyl pyrophosphoramide were also included in the tests. This paper reports on studies made to evaluate the killing efficiency and lasting qualities of lethal residues from these insecticides at certain dosages. Three insecticides; namely, DDT, methoxychlor and lindane are frequently recommended for Japanese beetle control. For that reason summaries of results obtained with them are included in the tables for comparative purposes.

METHODS.—Both the killing properties of the insecticides, as well as the lasting lethal effects from their residues were studied in the laboratory. Healthy beetles were collected from the field each day and 100 beetles were used in each test with 10 replications. In evaluating killing efficiency for the different insecticides, several different dosage levels were employed. Beetles were treated by spraying them directly and lightly with a hand

Table 2.—Comparative residual properties of some newer organic insecticides against the Japanese beetle.

Formulation of Insecticides	Dosage-Active Ingredients in Lbs. per 100 Gal.	% Kill from Residues Field Sprayed Leaves				% Kill from Residues Laboratory Sprayed Leaves				Check
		Fresh	1 Day	2 Days	3 Days	Fresh	1 Day	2 Days	3 Days	% Alive
Dilan emul. 25% 2-nitro-1, 1-bis (p.-chlorophenyl) propane +2-nitro-1, 1-bis (p.-chlorophenyl) butane	0.25	96	97	67	24	90	90	86	74	100
NPD emul. 85% Tetra-n-propyl dithienopyrophosphate	3.0	100	100	51	18	100	100	80	52	100
Malathon emul. 50% 0,0-dimethyl dithiophosphate of diethyl mercaptosuccenate	1.0	72	62	47	37	92	67	65	25	100
EPN, W. P. 27% Ethyl'p-nitrophenyl thiono benzene phosphate	3.0	100	89	50	6	100	100	87	11	100
Metacide emul. 6.3% Parathion+Dimethyl homologue	0.167	96	83	57	11	100	100	100	100	100
Systox emul. 32.1% Trialkyl thiophosphate	0.25	69	50	59	4	70	63	62	6	100
Lindane emul. 20%	0.167	73	37	12	9	93	70	61	25	100
DDT emul. 30%	1.0	—	—	—	—		96	100	88	99

atomizer. The beetles were then immediately transferred to absorbent paper towels where they were dried. Then they were caged on fresh grape leaves to feed. Residual toxicity was studied by exposing beetles on grape leaves that had been sprayed in the field as well as n the laboratory. Field treated leaves (on the vine) were harvested at stated intervals (Table 2) and brought to the laboratory where they were kept green by inserting vine stems with the leaves attached in bottles containing water. Test beetles were then confined to individual leaves, spread flat on a table surface by inverting a wide-mouth glass jar over each leaf so placed as to hold the leaf flat. The beetles received aeration through a hole in the bottom (top when inverted) of the jar. All dosages for insecticide are expressed in pounds of actual active ingredient in 100 gal. of water.

RESULTS.—Tables 1 and 2 give a summary of the results obtained in the tests designed to evaluate the several chemicals. All of the insecticides under test were found to be toxic to the Japanese beetle. All with the exception of the two so-called plant systemics, Systox and Octomethyl pyrophosphoramide killed beetles quite satisfactorily when used at the proper dosage level and were apparently safe on grape foliage. The systemics when employed as such did not appear to have value in killing beetles feeding on the foliage. It was possible to use them at dosage levels that were effective for killing beetles when they were applied directly to the insect as sprays. The knockdown rate was relatively slow and kills from ageing residues fell sharply after the second day in the case of Systox. Residual studies were not made with octomethyl pyrophosphoramide.

Dilan was found to be highly effective as an insecticide for controlling the Japanese beetle. It gave a very fast knockdown and left residual deposits that lasted up to seven days in a number of instances when applied to foliage at a dilution of 1 part of 25 per cent emulsion in 600 parts of water. In field spraying complete kills were obtained at dilutions of 1 part of the emulsion in 800 parts of water. Satisfactory kills have been obtained with dilutions as weak as 1 part in 1,600 parts of water. For commercial operations in which residual deposits must be taken into consideration, and on which weathering has a tremendous influence, a dilution of 1 part of the emulsion in 600 parts of water should probably be recommended. Laboratory studies, table 2, indicated that residual action on foliage sprayed with .25 pound of actual chemical in 100 gallons of water (approximately 1 part of the 25 per cent emulsion in 800 parts of water) fell rapidly after the second day.

Malathon also gave outstanding performance in killing Japanese beetles. One hundred per cent kills were obtained in sprays containing from 1 to 3 pounds of

the actual chemical in 100 gallons of water. At the dosages tested, initial knockdowns were much slower than those obtained from parathion and the related insecticide, known as Metacide, but ultimate kills appeared to be must as good. Residual kills from this insecticide were not fully tested. They were not outstanding when used at the 1 pound dosage level. General observations indicated that they were of relatively short duration. For field spraying indications are that at least 1.5 pounds of the actual chemical should be used in 100 gallons of water. Two and three pounds gave a much faster knockdown.

EPN-300 and NPD which are phosphorus base insecticides appeared efficient for killing the Japanese beetle at dosage levels of 2 and 3 pounds of the actual chemical in 100 gallons of water. They seem to be about equally effective, pound per pound. However, when the rate of knockdown is considered, EPN appears to be somewhat more effective. Like malathon, kills from residues fell rapidly as they aged. The residues were relatively ineffective after the second day.

The insecticide Metacide, which consists of parathion and its dimethyl homologue, was the most effective of the phosphorus base insecticides included in these tests. Sprays containing as little as .0625 of a pound of the actual chemical in 100 gallons of water gave complete knockdown and kills within 1 hour. But, as with the other phosphorus base insecticides, discussed in this paper, the spray residues from metacide were ineffective for killing any large number of beetles after an elapse of from two to three days.

Systox and octomethyl pyrophosphoramide were other phosphorus base insecticides tested. They are usually employed as systemic plant insecticides. Grapevines were heavily sprayed with these two materials at a dilution consisting of 1 quart of insecticide in 100 gallons of water. After a 5-day waiting period leaves were taken into the laboratory, kept fresh in water and beetles put on them to feed. After a twenty-four hour feeding period the beetles had consumed on the average, an area of 3.3 square inches per leaf for each ten beetles under the test. Mortality was insignificant; eight per cent being the maximum in any given test. This indicated that if the chemicals were absorbed and translocated successfully, there was not a sufficient amount present to kill Japanese beetles, or the time allotted for feeding was not sufficient to provide a lethal dose. Tests in which these chemicals were sprayed directly upon beetles showed that both of the insecticides were toxic to beetles. At high dosage levels (tables 1 and 2) both chemicals produced a 100 per cent kill. However, they were slow in knockdown. Systox was more effective than octomethyl pyrophosphoramide.

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ENTOMOLOGICAL SOCIETY OF CANADA
ANNUAL MEETING, VICTORIA B. C.,

October 19–21

Variations in Susceptibility of Citrus-Infesting Coccids to Parasitization[1]

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Diaspine and lecanine scale insects and mealybugs usually are without protection from parasitization, all developmental stages being particularly vulnerable to attack by entomophagous insects. The susceptibility of the group as a whole to parasitization is correlated with a marked overlapping of parasitization, a single host species being occasionally parasitized by two or more parasite species and differing in susceptibility to these species.

CITRUS-INFESTING COCCIDS SUBJECTED TO BIOLOGICAL CONTROL.—Biological control work with coccids is facilitated by the sedentary habits of the species and by their exposure to view (Smith 1929). They are readily collected in their native habitats, and the release of their natural enemies in new habitats is comparatively easy. Usually, hosts and parasites are easily propagated in the laboratory. The importance of biological control of coccids in California has been accentuated by the fact that the serious insect pests of citrus are practically all scale insects, none of which is native to California.

Most of the natural enemies of coccids introduced into California during the past 50 years consist of hymenopterous parasites. This is in marked contrast to the introductions prior to 1901, which consisted almost entirely of coccinellid predators. The introduction of the hymenopterous parasites was necessary because of the failure of the predators to effect commercial control. Citrus-infesting coccids now under unlimited commercial control in California by parasites are the soft scale, *Coccus hesperidum* L., by *Metaphycus luteolus* (Timb.); the nigra scale, *Saissetia nigra* (Nietn.), by *Metaphycus helvolus* (Comp.); and the citrophilus mealybug, *Pseudococcus gahani* Green, by *Tetracnemus pretiosus* Timb.

Citrus-infesting coccids under area-limited commercial control by parasites are the cottony-cushion scale, *Icerya purchasi* Mask., by *Cryptochaetum iceryae* (Will.); the citrus mealybug, *Pseudococcus citri* (Risso), by *Leptomastidea abnormis* (Gir.); the long-tailed mealybug,

[1] Paper of the University of California Citrus Experiment Station, Riverside, California.

Table 1.—Hymenopterous parasites propagated on *Saissetia* species, 1924–1952.[1]

PARASITE SPECIES	PLACE OF COLLECTION	COLLECTOR	DATE OF COLLECTION	PARASITES IN CALIFORNIA		LABORATORY HOST SPECIES
				Released	Established	
Scutellista cyanea Motsch.	S. Africa	C. P. Lounsbury	1901	Yes	Yes	*Saissetia* spp.
Metaphycus lounsburyi (How.)	Australia	E. J. Vosler	1918	Yes	Yes	*S. oleae*
Lecaniobius cockerelli Ashm.	British W.I.	G. F. Moznette	1922	Yes	No	*Saissetia* spp.
	Puerto Rico	K. Bartlett	1940			
Anysis saissetiae (Ashm.)[2]	China	F. Silvestri	1924	Yes	No	*Saissetia* spp.
Coccophagus capensis Comp.	S. Africa	E. W. Rust	1924	Yes	Yes	*S. oleae*
Coccophagus trifasciatus Comp.	S. Africa	E. W. Rust	1924	Yes	Yes	*S. oleae*
Euxanthellus sp.[2]	S. Africa	E. W. Rust	1924	No	No	*Saissetia* spp.
Coccophagus baldassarii Comp.[2]	Eritrea	H. Compere	1930	No	No	*Saissetia* spp.
Coccophagus caridei Brèthes	Argentina	H. Compere	1934	Yes	No	*Saissetia* spp.
Lecaniobius utilis Comp.	Brazil	H. Compere	1934	Yes	?	*Saissetia* spp.
Aneristus ceroplastae How.	Hawaii	D. T. Fullaway	1936	Yes	No	*Saissetia* spp.
	Formosa	T. C. Maa	1951			
Coccophagus cowperi Gir.	S. Africa	H. Compere	1937	Yes	Yes	*Saissetia* spp.
Coccophagus pulvinariae Comp.	S. Africa	H. Compere	1937	Yes	Yes	*Saissetia* spp.
Coccophagus rusti Comp.	S. Africa	H. Compere	1937	Yes	Yes	*S. oleae*
Diversinervus smithi Comp.	S. Africa	H. Compere	1937	Yes	No	*S. oleae*
Encyrtus fuliginosus Comp.	S. Africa	H. Compere	1937	Yes	No	*S. oleae*
Metaphycus helvolus (Comp.)	S. Africa	H. Compere	1937	Yes	Yes	*Saissetia* spp.
Metaphycus stanleyi Comp.	S. Africa	H. Compere	1937	Yes	Yes	*Saissetia* spp.
Mesopeltis sp.	Uganda	H. Compere	1937	Yes	No	*Saissetia* spp.
Microterys saissetiae Comp.	Uganda	H. Compere	1937	Yes	No	*Saissetia* spp.
Coccidoxenus niloticus Comp.	Kenya	H. Compere	1937	Yes	No	*Saissetia* spp.
Encyrtus sp.	Kenya	H. Compere	1948	No	No	*S. oleae*
Coccophagus yoshidae Nak.	Japan	J. L. Gressitt	1951	Yes	No	*S. oleae*
Coccophagus japonicus Comp.	Japan	J. L. Gressitt	1951	Yes	No	*S. oleae*

[1] Propagation by S. E. Flanders, except as indicated.
[2] Propagated only by Harold Compere.

Pseudococcus adonidum (L.), by *Anarhopus sydneyensis* Timb.; the yellow scale, *Aonidiella citrina* (Coq.), by *Comperiella bifasciata* How.; the red scale, *Aonidiella aurantii* (Mask.), by *Aphytis chrysomphali* (Mercet); and the black scale, *Saissetia oleae* (Bern.), by *Metaphycus helvolus*.

In the period from 1924 to 1952, 24 species of introduced parasitic Hymenoptera have been cultured on black scale (Table 1); 23 species on citrus-infesting diaspine scale insects, California red scale, yellow, purple, and Glover scales (Table 2); and 16 species on citrus-feeding mealybugs (Table 3). This work has emphasized the fact that susceptibility to parasitization is a highly variable phenomenon.

HOST-PARASITE RELATIONS.—It is possible to analyze host-parasite relations from opposite points of view, that of the host and that of the parasite, the criteria being susceptibility to parasitization on the part of the host and host specificity on the part of the parasite. Under laboratory conditions the host list of a parasite tends to be larger, and the susceptibility of a host to parasitization tends to be greater, than under natural conditions.

It has been pointed out by Salt (1938) that the host specificity of a parasite is attained by four consecutive processes of host elimination, namely, by (1) host habitat finding, (2) host finding, (3) host recognition and acceptance, and (4) host suitability; and that the first of these processes removes the largest number of species from the potential host list.

The susceptibility of a host to parasitization is determined by the following factors (Flanders 1940): (1) the extent to which the host habitat is frequented by the parasite, (2) the proportion of the host population exposed to attack, (3) the degree to which the host stimulates egg deposition by the parasite, and (4) the proportion of parasitized individuals suitable for the complete development of the parasite.

As pointed out by the writer (Flanders 1937) the susceptibility of various hosts to successful parasitic attack may not be correlated with phylogenetic relations, but only with their suitability as food. Many parasitic Hymenoptera oviposit in any insect that happens to be suitably located in the type of plant which they habitually frequent (Gahan 1933).

Usually, the insect enemies of an insect species form a group of more or less unrelated organisms having little or no correlation with the systematic position of the host, but the natural enemies of the Homoptera consist, for the most part, of highly specialized genera in the hymenopterous families Aphelinidae, Platygasteridae, and Encyrtidae.

TYPES OF INADEQUATE PARASITIZATION.—From the standpoint of host-parasite balance or equilibrium, parasitization of a scale insect or mealybug under a particular environmental condition may be classified as adequate or inadequate. The degree of balance or "control" obtained by adequate parasitization may or may not be "commercial." With inadequate parasitization, balance such as occurs with the instances of biological control noted above is impossible because the host-parasite relation is incomplete.

An analysis of the host-parasite relations during the propagation of the parasites listed in tables 1, 2, and 3 revealed three type of inadequate parasitization designated herein as "occasional parasitism," "physiologically incomplete parasitism," and "ecologically incomplete parasitism."

The biologies of the red-scale-feeding parasites show that the suitability of California red scale for the reproduction of different parasitic species is highly variable, being complete for some and incomplete for others.

"Occasional parasitism" occurs with hosts that usually do not stimulate oviposition by the parasite, and that usually are not suitable for the development of its immature stages. This type of deficient parasitism is exhibited in relation to California red scale by *Casca* sp. "B," *Casca* sp. "F," and *Prospaltella* sp. "P."

"Physiologically incomplete parasitism" occurs when in otherwise healthy hosts the parasite suffers a high host-determined mortality during its immatuer stages. In this type of parasitization the host stimulates maximum oviposition by the parasite. Examples of this type of inadequate parasitism include four of the red-scale-feeding parasites, *Habrolepis rouxi* Comp., *Physcus* sp. "D," *Prospaltella* sp. "G," and the Japanese race of *Comperiella bifasciata*. The low suitability of the red scale for the develop-

Table 2.—Hymenopterous parasites propagated on citrus-infesting diaspine insects, 1932–1952.[1]

Parasite Species	Place of Collection	Collector	Date of Collection	Parasites in California		Laboratory Host Species
				Released	Established	
Aphytis chrysomphali (Mercet)[2]	California	———	—	—	Yes	*Aonidiella aurantii*
Comperiella bifasciata How. (Japanese race)	Japan	F. Silvestri	1925	Yes	Yes	*A. citrina*
Habrolepis rouxi Comp.	S. Africa	H. Compere	1937	Yes	Yes	*Aonidiella* spp.
Comperiella bifasciata How. (Chinese race)	China	J. L. Gressitt	1941	Yes	Yes	*A. aurantii* *A. citrina*
Aspidiotiphagus citrinus (Craw.)	China	J. L. Gressitt	1947	Yes	?	*A. aurantii* and other diaspine scales
Aphytis sp. "A"	China	J. L. Gressitt	1947	Yes	Yes	*A. aurantii* and other diaspine scales
Anabrolepis sp.	China	J. L. Gressitt	1948	No	No	*A. aurantii*
Aphytis sp. "X"	China	J. L. Gressitt	1948	Yes	Yes	*Lepidosaphes beckii*
Casca sp. "C"	China	J. L. Gressitt	1948	Yes	No	*Aonidiella* spp.
Prospaltella elongato Dozier	China	J. L. Gressitt	1948	Yes	Yes	*L. gloverii* (Pack.)
Aspidiotiphagus lounsburyi B. & P.	Florida	Paul DeBach	1949	Yes	?	*Aonidiella* spp.
	Formosa	T. C. Maa	1951			
Prospaltella perniciosi Tower	Formosa	T. C. Maa	1949	Yes	Yes	*Aonidiella* spp.
Casca chinensis How.[3]	China	J. L. Gressitt	1949	Yes	No	*A. aurantii*
Casca sp. "B"	China	J. L. Gressitt	1949	No	No	*A. aurantii*
Physcus sp. "B"	Formosa	T. C. Maa	1950	Yes	Yes	*Lepidosaphes beckii*
Aphytis sp. "B"	Formosa	T. C. Maa	1951	Yes	?	*A. aurantii*
Physcus sp. "D"	Pakistan	A. M. Boyce	1951	No	No	*A. aurantii*
Prospaltella sp. "P"	Formosa	T. C. Maa	1951	No	No	*A. aurantii*
Aphytis sp. "F"	Formosa	T. C. Maa	1952	?	?	*A. aurantii*
Casca sp. "F"	Formosa	T. C. Maa	1952	No	No	*A. citrina, A. aurantii*
Prospaltella sp. "G"	Formosa	T. C. Maa	1952	No	No	*A. aurantii, A. citrina*
Prospaltella sp. "K"	Formosa	T. C. Maa	1952	No	No	*A. citrina*
Prospaltella aurantii How.	Florida	S. E. Flanders	1952	No	No	*A. citrina*

[1] Propagation by S. E. Flanders.
[2] This species probably accidentally introduced into California with its host *Aonidiella aurantii*.
[3] Propagated for only one generation because the host of the male is unknown.

ment of *H. rouxi* is acquired only by feeding on sago palm. With the other parasites the low suitability of the host is inherent.

"Ecologically incomplete parasitism" occurs when the number of host habitats in which a highly suitable host is susceptible to attack is less than the total number of habitats occupied in common by this host and its parasite. Biological control workers are well aware of the fact that under field conditions certain hymenopterous parasites do not successfully parasitize host species that are physiologically suitable. *Casca* sp. "C," for example, propagates readily on California red scale under laboratory conditions, but not under California field conditions. This appears to be the case, also, with the red scale parasite *Aphytis* sp. "B." The purple scale and the parasite *Aphytis diaspidis* (How.) are ecologically associated in southern California, but the parasite successfully parasitizes purple scale only in the laboratory.

This variation in susceptibility occurs

Table 3.—Hymenopterous parasites propagated on citrus-infesting mealybugs (*Pseudococcus* spp.), 1927–1952.[1]

Parasite Species	Place of Collection	Collector	Date of Collection	Parasites in California		Laboratory Host Species
				Released	Established	
Leptomastidea abnormis Gir.)	Sicily	H. L. Viereck	1914	Yes	Yes	*P. citri*
Coccophagus gurneyi Comp.	Australia	H. Compere	1927	Yes	Yes	*P. gahani*
Tetracnemus pretiosus Timb.	Australia	H. Compere	1927	Yes	Yes	*P. gahani*
Anusoidea comperei Timb.[2]	Australia	H. Compere	1927	No	No	*P. gahani*
Anarhopus sydneyensis Timb.	Australia	Luke Gallard	1933	Yes	Yes	*P. longispinus*
Leptomastix dactylopii How.	Brazil	H. Compere	1934	Yes	No	*P. citri*
Tetracnemus peregrinus Comp.	Brazil	H. Compere	1934	Yes	Yes	*P. longispinus*
Aenasius paulistus Comp.	Brazil	H. Compere	1934	No	No	*P. maritimus*
Anagyrus pseudococci Comp.	Argentina	H. Compere	1934	Yes	No	*P. citri*
Anagyrus fusciventris (Gir.)	Hawaii	Walter Carter	1936	Yes	Yes	*P. longispinus*
Anagyrus kivuensis Comp.	Kenya	H. Compere	1948	Yes	No	*P. citri*
Tropidophryne melvillei Comp.	Kenya	H. Compere	1948	Yes	No	*P. citri*
Pauridea peregrina Timb.	China	J. L. Gressit	1950	Yes	Yes?	*P. citri*
Allotropa sp.	China	J. L. Gressit	1950	Yes	No?	*P. citri*
Coccophagus sp.	Hong Kong	S. K. Cheng	1950	No	No	*P. citri*
Pseudaphycus sp.	Formosa	T. C. Maa	1950	No	No	*P. citri*

[1] Propagation by S. E. Flanders, except as indicated.
[2] Propagated only by Harold Compere.

also with the black scale (Table 1) and the citrus-feeding mealybugs (Table 3) and their parasites. Studies by Bess (1939) on the susceptibility of *Pseudococcus citri*, *P. gahani*, *P. adonidum*, and *P. maritimus* to the development of the larvae of *Coccophagus gurneyi* and *Leptomastix dactylopii* show that the host species vary from no susceptibility to complete susceptibility. During the period 1937–41, ten species of parasites introduced into California from Africa were propagated by the thousands on black scale in the laboratory. After being released in southern California in large numbers, five of these species became established. Significantly, one of the established species does not reproduce on black scale but reproduces on a related scale insect, *Saissetia nigra*.

The mealybug parasites *Anagyrus kivuensis* Comp., *Tropidophyrne melvillei* Comp., and *Leptomastix dactylopii* have been released in numbers against *Pseudococcus citri*, but lack of establishment shows that the host-parasite relations are ecologically incomplete. *L. dactylopii* has been released at the rate of over 10 million per year for the past 15 years by the Associates Insectary of Santa Paula, California, without establishment.

An interesting relation occurred with a *Coccophagus* species from Hong Kong. This parasite reproduced under laboratory conditions on *Pseudococcus citri* only when the latter was part of a mixed culture of *P. citri* and *P. maritimus* (Ehrh.), but did not reproduce on a pure culture of either *P. maritimus* or *P. citri*. Apparently with this parasite the presence of *P. maritimus* is necessary for oviposition in *P. citri*.

A similar situation may have occurred in experiments by Bess (1939) in obtaining oviposition by *Leptomastix dactylopii* in *Pseudococcus adonidum*, *P. gahani*, and *P. maritimus* where cultures of these hosts were mixed with *P. citri*.

Anarhopus sydneyensis, unlike most parasites of coccids, reproduces on *Pseudococcus longispinus* more readily under field conditions than it does in the laboratory.

SUMMARY.—The propagation of hymenopterous parasites of diaspine and lecanine insects and mealybugs during the past twenty years has revealed marked variations in susceptibility of these hosts to parasitization. Host-parasite relations within the limits of a particular environment are either adequate or inadequate for the establishment of host-parasite equilibrium. Inadequate parasitism may be "occasional," "physiologically incomplete," or "ecologically incomplete."

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Army Insect Control Operations in the Far East

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Although the insect control operations of the Far East Command were conducted in the Orient, leading one to expect something rather special in the way of vectors and problems, there was actually nothing particularly exotic about the procedures involved.

In 1946, the control of insects acting as disease vectors, affecting the morale of troops, civilian employees, and dependents, or attacking property and supplies, in Far East installations, became the duty of the Corps of Engineers. Late in that year, civilian crews were established by the Post Engineers and control operations were commenced. By 1947, each Army area had been placed under the direction of an entomologist.[2] There were already entomologists of the Army Medical Service assigned to investigations in several of the areas,[3] and surveys to determine the extent of the insect populations, and to evaluate the effectiveness of the control, continued to be conducted by the Medical Service wherever there remained a sufficient number of survey personnel.

At all levels the two sections of the Army technical services worked together as one team, Medical Service personnel being often utilized to give supervision to newly-established crews, while, in other localities, because of the lack of Medical Service personnel, it was necesary to train Post Engineer employees to carry on the survey. This coördination was desirable inasmuch as the combined weight of the Surgeon and the Engineer was sometimes required to develop a program of suitable operations.

These operations were further hampered by the fact that equipment was normally made up of the left-overs from World War II. The effort to obtain effective results with these sub-standard or deteriorated pieces of equipment gave considerable exercise to the ingenuities of Americans and local civilians alike. Many of the makeshift modifications and improvisations which originated in these forward areas are now under study at the Engineer Research and Development Laboratories at Fort Belvoir, Virginia, in connection with a program for the development of improved equipment.

At the beginning, all crews were composed of both enlisted men and local civilian controllers; in some cases, the enlisted men who were used as supervisors were trained technicians of Malaria Survey and Control Detachments. In most cases, however, they were casuals.

Local civilians were also recruited and trained as supervisors, bilingual schools being held every 6 months for this purpose, which military and civilian personnel, both American and local, attended. We were fortunate in securing some local men of collegiate training, some with experience in health departments, some ex-Philippine Scout technicians, and a great many others who were untrained but intelligent, interested, and energetic. In Guam, the Philippines, and Okinawa, the civilian supervisors eventually replaced the military completely. The entomologist of each area made continual trips from post to post, advising the men and stimulating local support for the program, and in the end there resulted a compact group of alert and capable enlisted and civilian supervisors. The continual reduction of insect-borne disease rates among Army personnel in all areas, and the improvement in local sanitation in non-military zones surrounding Army posts, were notable and this was due in no small measure to the high calibre of these men, American, Filipino, and Japanese.

Problems of insect control in Japan, Korea, Okinawa, the Philippines, and Guam were widely different in many respects, but at the beginning all had in common the war-torn conditions of the areas and the temporary and hasty character of the military constructions. As these conditions gradually disappeared, and more permanent and improved situa-

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tions took their places, it became evident that there was nothing radically different in the situation from that in the various parts of the United States.

In Japan, it is true, some of the mosquitoes breed in the flower urns and incense-stick boxes which are to be found before each stone in Buddhist and in Shinto cemeteries. The numerous water receptacles for many purposes, which are important parts of all Japanese landscape gardens, are also prolific sources of mosquitoes; however, the breeding of most of the vectors of diseases other than malaria occurs in such universal and ordinary places as privies, ditches, and carelessly discarded tin cans. Often beer cans alone, tossed into ditches and jungle growth, or, as in one case in Korea, into barbed wire entanglements around the area, produced larger numbers of mosquitoes than did all the natural sites in the vicinity.

Rice paddies, the first place to be suspected of mosquito breeding by newcomers to the area, were found to be a relatively far less important site of breeding than had been expected; nevertheless, the population breeding in rice paddies in Korea and Okinawa, for instance, is of prime importance. In all areas except the Philippines and the southern Ryukyus the vector of malaria is a paddy breeder, and some of the culcine mosquitoes also commence the season as paddy breeders.

Malaria was, of course, the primary target for control measures in all areas. Although the disease had diminished almost to the vanishing point in Japan, so that it was not considered a problem, actually there were a number of cases each year among the Japanese. The threat continued to exist, that, if anophelines were not controlled, there might be renewed outbreaks of the disease, especially around camps where patients from Korea were hospitalized. The carrier in Japan, Okinawa, and Korea is *Anopheles sinensis*. In the southern Ryukyus, *A. minimus* and *A. ohami* were added vectors, somewhat replacing *A. sinensis* in population density. In the Philippines, the primary vector is *A. minimus* var. *flavirostris*.

In Okinawa and Korea, *Anopheles sinensis* was far more of a problem than it was in Japan, both because the malaria rate among Okinawans, Filipinos, and Americans was higher than in Japan, and also because the Army installations were more intimately surrounded by the paddies. An unorthodox control method which worked quite well, although it was somewhat unscientific, was to issue DDT 10 per cent dust in small quantities to individual farmers, who applied it from handkerchiefs held in the hand in such a way as to create a bag. These were shaken by the farmers over their paddies and it seemed fairly sure that they were shaken surreptitiously also over the nearby cabbage patches. Control of *A. sinensis*, *C. tritaeniorhynchus*—and *Pieris rapae*—was good in all cases.

In the Philippines a stream breeder, *Anopheles minimus* var. *flavirostris*, is the malaria carrier, and neither *A. sinensis* nor *A. vagus limosus*, though abundant, is considered to be a vector. *A. minimus* is often said to breed only at altitudes below 2000 feet and in the eddies of clear, flowing water; however, many of the finest residential sections of Manila were along not-too-clear streams in which abundant breeding of *A. minimus* was found, and so many cases of malaria showed up in the military patrons of one downtown nightspot that we were asked to include it in our routine control. In the streams around the mountain city of Baguio, *A. maculatus*, a possible carrier, was found in considerable quantities, and we did, in fact, have a number of cases of malaria among persons who had never visited the lowlands. *A. minimus* was also found breeding occasionally in shower drains and in animal footprints, and the Bureau of Health found it in a drain in the Chinese cemetery in Manila.

Various of the so-called "pest" mosquitoes are implicated in the transmission of serious diseases, and their control is, therefore, far from being merely a matter of comfort. *Aëdes albopictus*, the vector of dengue, is common on Saipan and in the Philippines, particularly in Manila; it occurs, though somewhat less extensively, also on Okinawa and in Japan, where epidemics of dengue have occurred in the past. It has become widely established on Guam since 1947. *Aë. aegypti*, the vector of edngue on Guam in the epidemic of 1944, was apparently eradicated at that time, and careful surveys since have failed to uncover any evidence of its return.

In Okinawa, the use of C-47 airplanes

was a routine of mosquito control measure from 1945–47. This form of airplane spraying was suspended in 1947, and was not resumed because of the damage being done to rice and even to pine trees, and because of the lack of effectiveness resulting from poor pilot techniques. Population surveys showed that the claimed reduction of mosquitoes and of flies was purely imaginary. The use of liaison-type planes, more suited to the terrain was, on the other hand, found to be a most desirable component of the mosquito control program.

In this project, close ocordination between the Air Force pilots and the Ground Forces entomologists was maintained at a local level. Counts of mosquito populations told us with great accuracy when the airplanes were out of use or were being improperly operated.

DDT 10 per cent dust was used during the late-season operations in order to penetrate fully-grown rice, and helicopters, equipped with only an operator cranking a hand rotary duster, were used effectively in ravines large enough to contain a paddy but too small to be entered by an L-5. Similar airplane operations with liaison-type planes have been notably effective in Korea. No airplane spraying or dusting was permitted in Japan, of course, because of the danger to sericulture. This caused no hardship since the mosquito breeding sites in virtually all areas were inaccessible to airplane-distributed sprays.

Extensive tests in conjunction with the Philippine Bureau of Health were conducted during 1947–48 in an attempt to evaluate the use of airplane sprays against *Anopheles minimus* larvae and of fog generators against larvae and adults of *A. minimus*, *A. sinesis*, *C. fatigans* (=*quinquefasciatus*), and *Aëdes albopictus*. Breeding streams in the vicinity of the Tala Laboratory of the Bureau, on land which was kindly lent for the purpose by the Seminary of the Ataneo de Manila, were used for many of the tests. Animal-baited traps containing carabao were constructed and adult catches were counted daily by men working under Dr. A. Ejercito, Philippine Bureau of Health, and Mr. Cornelio Urbino, Entomologist in charge of the Tala Laboratory. Larval counts were also made and sprays were applied at normal rates of one quart per acre of 5 per cent DDT in diesel oil, applications being made by an L-5 plane flying at a 50-foot altitude and about 75 mph., between the hours of 5 and 6 A.M., over ground which was covered with *cogon* and *talahib* grasses. After the grass was burnt over, the same area was again tested. It was found uniformly that the sprays failed to penetrate the heavy grasses but that kills were excellent when the grass was cleared or burnt. Dusts, however, gave good larval control at all periods. Adult populations of *A. minimus*, which normally rest in the cover at the breeding sites during the day, were not affected by sprays except when the cover was reduced.

Thermal fog generators, both modified Army smoke generators and a well-known commercial brand, were used in a series of some 15 tests. Fogging was done both in rural areas and in the city, the breeding areas in each location being somewhat concealed, in the country by bamboo thickets and in the city by rubble. DDT 5 to 20 per cent solutions in diesel oil with added xylene and fog oil, were used for the insecticide. Adults in cages were used as test insects and counts of larvae and of biting adults were made as an index of natural populations. The results were uniformly disappointing.

Because of these unproductive results in tests with fog generators, they were little used in the Philippines. On Okinawa and in Japan, some fogging was done with a semi-thermal type of fog generator manufactured in Japan, which gave a very good particle size and was fairly effective in adult applications. On Guam, a converted Navy-type smoke generator, which gave a fairly wet mist, was utilized for adulticiding at dusk, but again, the results were not satisfactory. The establishment of *Aë. albopictus* and *A. subpictus* on Guam is thought to be traceable to excessive dependency on foggers. The use of foggers was therefore not emphasized, inasmuch as the people were already far too prone to wish the work done by foggers and airplanes rather than to make the more difficult efforts to support sanitation and basic treatments.

The basic treatments were the same in all parts of the Far East Command. To begin with, the walls of the dwellings, their screens (if any), and bed nets (if any), were sprayed with 5 per cent DDT

"residual spray," usually in kerosene solvent, at intervals of 60 to 90 days. As nearly as possible, 1 quart of spray was applied to 250 square feet of wall surface. Spraying was done on a scheduled basis and also at extra intervals on request. Mess hall screens were treated monthly.

After interior residual spraying was accomplished, all breeding sources discovered within the military area were either eliminated or treated to prevent any breeding from occurring. DDT 10 per cent dust was applied lightly at about 2 lbs. per acre to all shrubbery and outbuildings in the area, and to buildings and foliage in the zone surrounding the area as far out as was feasible. Nearby ditches, privies, and night-soil pits, *etc.*, were sprayed.

Finally, power dusters and fog or mist machines which gave a wet cloud of particles limited to the range of 30 to 120 microns and averaging about 65 microns, were used throughout the nearby surrounding districts. Obviously, these latter two operations required the close cooperation of the local municipal, prefectural or provincial control organizations and this cooperation was invariably forthcoming, albeit with somewhat fluctuating degrees of efficacy.

Survey methods practiced by the Malaria Survey Detachments and the Engineer civilian employees assigned to survey, were the ones commonly practiced elsewhere. In paddies and streams, ten dips were taken in five locations, giving a total of fifty dips, and the results were expressed as the average count per dip. Larval check stations were established as areas, but not as specific sites, and the men were taught to vary their dipping within a certain length of stream bed or a certain cluster of paddies, in order to avoid sampling the same locations over and over, and also to avoid failing to search for areas which might have escaped treatment. Dipper techniques were also varied from sudden scoops to slow immersions and gentle skimmings, and, of course, preliminary evaluations were made by eye, particularly with culicines which were likely to sink to the bottom for protracted periods. Pipettes were used to sample breeding sites which were too small or shallow to be dipped.

Not only survey teams, but control crews also, were trained to search for cans, tubs, pits, abandoned wells and privies, obscured ditches, and other places which might otherwise have been overlooked by the survey. In many places, survey teams were accompanied by control "scouts" who treated or marked for immediate attention any spots which were discovered to harbor previously uncontrolled breeding.

Survey was an essential complement to control, and saved hundreds of man hours and much material by demonstrating the effectiveness of treatments, making it possible to gauge the amount of time, materials, and manpower needed to effect control in each area at each period. Often it was seen that blanket control would be a waste of effort while at other times residual effects of several successive treatments would enable the control crew to skip treatment for several weeks.

Other insects, in addition to mosquitoes, were also of importance in the control programs. Flies, breeding principally in garbage or improperly closed privies and army latrines, were suspected as carriers of dysenteries and round worms. *Musca*, *Chrysomyia*, and *Lucilia* were common wherever control was lax. The eradication of *C. megacephala*, *C. bezziana*, and *L. caesar* was especially difficult since they bred in places not susceptible to good control and seemed always to alight either directly on the food or on the floor, neither of which could be effectively sprayed with DDT. Garbage separation points and pig farms run by contractors were therefore treated with 5 per cent residual spray made from a 50 per cent wettable DDT powder, and clean-up operations were required of the owners. Where the Army provided for its own waste disposal, on Okinawa, Guam, and in the Philippines, sanitary land fills were put into operation and open or covered dumps were prohibited.

Fills were operated in sand, coral, mud, adobe (a soft stone in the Philippines), and other trying situations, but always with eventual success and complete elimination of fly breeding. Since adult flies were found to ride in great numbers for distances as far as 10 miles, all Army or contractors' trucks which went to fly-breeding areas were routinely sprayed each week with 5 per cent DDT residual spray in kerosene. Spray was applied until the surface was wet, but not past the

run-off point, which is to say at a rate of about 1 quart per 250 square feet.

Mites, carriers of scrub typhus or *tsutsugamushi* fever in parts of Japan, have been controlled by repellents and by rodent eradication, usually under Medical Service direction. Occasionally, rodent control and area dusting were accomplished by Post Engineer crews in lieu of the bulldozing of whole maneuver areas, often several miles square, which was otherwise demanded. Control was apparently satisfactory.

An imaginary disease, which was nevertheless of considerable concern to the Medical Service in Manila was caused by the presence in large stocks of flour of the usual flour insects, including especially large populations of *Tribolium confusum*. This condition arose from the fact that flour stocks were necessarily held under the most adverse of storage conditions and consisted of mixed stocks collected from many parts of the former combat areas of the Pacific. Fumigation materials were scanty and the fumigation which was arranged required the use of cyanide in the form of cyaneggs, cyanodust, and discoids in combination. The warehouses were open-sided, there was little facility for refrigeration and the supply of appropriate canisters for gas masks was insufficient. Under these conditions, a fumigation should not ordinarily have been attempted, but in view of the moral factor involved, the warehouse was covered with tarpaulins and treated, and good control was obtained.

Following fumigation, 10 per cent DDT dust was applied lightly over the surfaces of rice and flour bags and this was repeated after an interval of six weeks. As in Japan, where *Sitophilus oryzae* and *Tribolium confusum* were common in flour and in rice, the dust prevented any further reinfestation.

Disease bearers were not, of course, the only insects of importance whose eradication was desired by the Army. Morale-destroying insects were also of interest. The black-fly *Eusimulium equinum*, was a severe pest for short periods and in limited locales throughout Japan, and caused various psychosomatic disorders. Its control was effected by spraying streams lightly with DDT emulsion, transverse to the flow and well above the camp areas. Water treatment and sewage treatment plants, particularly the Balera Filter Plant near Manila, experienced outbreaks of *Psychoda alternata*, which was also controlled by emulsion, sprayed during back-flushing and also applied to the walls for adult control. *Chironomous dorsalis* was common in the drainage systems under buildings, such as hotels, which had been built in swampy areas.

Also of concern to the Engineers were shrubbery insects such as the "raincoat worm" or bagworm, the fall webworm (known as the "American moth" in Japan), various cerambycid tree borers, scale insects, termites, and powderpost beetles and other destroyers of property. In Manila, possibly due to the airplane spraying conducted with C-47's against the *Musca* and *Chrysomyia* flies which bred in the debris of the battle, and to later similar spray activities against mosquitoes in areas surrounding the city, hymenopterous parasites seemed to lose control of the populations of scale and mealybugs, and these became a severe pest on mango, hibiscus, poinsettia, croton, *etc.* Such pests were controlled both there and in Japan by 2 per cent sprays of DDT xylene emulsion.

Termites became a problem in the Philippines within two years of the end of the war. The mound builder, *Macrotermes gilvus* (Hagen) reared clay skyscrapers which pushed up against floors and acted both as unsteadying levers against the structure and as entryways for rats, while *Coptotermes vastator* Light, *etc.*, fed upon the wood of the building. Occasionally, control was called for by persons on whose mango trees nests were being built by *Nasutitermes luzonicus* (Oshima) and *Hospitalitermes luzonensis* Oshima although the termites did not cause any apparent injury. Control of the subterranean termite in the Philippines was not always possible by soil treatment inasmuch as the lower floor of the house frequently had cement or tile flooring which we disliked to destroy or damage. It was therefore decided to try breaking into the mud tunnels and blowing DDT 10 per cent dust into them in the manner suggested many years ago by David T. Fullaway for treatment of telegraph poles in Honolulu. This succeeded in completely wiping out the colonies and there-

after, whenever the soil was accessible, in addition to treating it with DDT (and later pentachlorophenol), dust was applied into any tunnels which were found to be in active use in the upper structure of the building.

Within 2 years of the commencement of occupancy of wooden buildings in Japan, termite injury began to necessitate extensive repairs and replacement of timbers. Many of these buildings were renovated Japanese Army barracks, others were hastily built by crews of rapidly recruited and as yet untrained workmen. The buildings were therefore often constructed too close to the ground, were inadequately shielded, or were otherwise conducive to termite and dry rot attack. *Leucotermes speratus* (Kolbe) and other species were found in buildings as far north as Sendai. In Kyushu, the southern island, termite feeding caused a severe structural damage to celotex-type insulation which was used as a subflooring on the seventh floor of a ferroconcrete building. In this case, the nest was located in 2-foot deep mounds of soil on the roof of the building, which had been placed there as part of the typical roof-garden common on buildings throughout Japan. Leakage from a "brook" and an artificial pond had maintained the moisture, and, in fact, excessive swabbing of the wooden parquet floor which lay over the celotex material had kept the moisture situation unusually favorable throughout. Control was gained, as in the Philippines, by dusting into the channels. Elsewhere, standard soil treatments and replacement of damage timbers with pentachlorophenol-treated timbers prevented further damage and in no case was there recurrence of infestation after treatment.

In Manila, the rattan and bamboo Malay-style Army chapel, which had a Nipa palm thatched roof, developed severe infestations of *Heterobostrychus aequalis* (Watrh.), *Dinoderus minutus* (F) in the wood, and of the pyralid moth *Herculia nigrivitta* (Wlkr.) in the thatch. These infestations all succumbed to a series of three weekly sprayings with 5 per cent DDT in kerosene, in which heavy rundown of solution into the crevices allowed the spray solution to soak into cut surfaces.

On several occasions pine trees of considerable antiquity and beauty in installations under Army control in Japan were attacked or threatened by bark beetles of various sorts, notably, *Myelophilus piniperda* L., in accordance with the advice of the foresters of the Japanese Government and of Drs. Kojima and Murayama of the Universities of Tokyo and Yamaguchi, who were then working with the Occupation Authorities, trees which had visibly commenced to die were felled and the bark burned. However, many trees showing only slight attack were sprayed heavily with 5 per cent suspensions of DDT in wettable powder, at about 5-week intervals from April through July. Yellowing or failing branch tips were carefully collected and burned. These sprayings were repeated on two successive years, after which no further damage by the beetles was observed. In the 3 years since completion of this project there has been no recurrence of injury, and examination of bark samples has not disclosed any fresh infestation among the several hundred trees in three locations in Tokyo, Saitama Ken, and Ibaraki Ken.

Ants and cockroaches were controlled by thorough applications of 10 per cent DDT dust to all cracks, crevices, and entries. DDT spray, alone, was not effective. A 2 per cent solution of chlordane, sprayed into cracks and behind baseboards, gave immediate kills and no repetition was needed for about 90 days.

No resistance to DDT by any insect except lice was noted in any area. Resistance by flies and roaches was reported several times, but investigation invariably showed that there had been a failure in the thoroughness of the applications. This led us to feel that the failure in Korea was also due to this cause, and it was with the greatest reluctance that actual resistance of the lice was at length acknowledged as being incontrovertible. Whether lack of resistance in flies, fleas, and roaches was due to the fact that thorough coverage allowed very few survivors to carry on the population or whether the continual influx of untreated populations kept non-resistance alive in the strain, is not known. However, the virtual blanketing of Tokyo with BHC dust by Parks Department crews who were after the Fall Web-worm, introduced since the

war, seemed to have a depressant effect upon the populations of mosquitoes and flies in the metropolitan area, and there was no resistance to BHC or DDT evident up to the end of 1951.

Because of the distance from which supplies were received, crews were often short of materials and were forced to exercise great economy. The Army use of DDT was, of course, very thorough, but it was not a wasteful operation. It became evident, therefore, that in all these areas control, though tedious, was an entirely feasible operation.

SUMMARY.—In 1946 control of insects acting as disease vectors, damaging morale, or attacking property and supplies, became the duty of the Corps of Engineers in the Far East Command. Survey of insect populations was conducted by the Medical Service. These worked in close coordination and frequently combined functions. Enlisted men and local civilian crews were utilized. Schools were held to train personnel, and a high calibre of men was secured.

Disease vectors included *Anopheles sinensis*, *A. minimus* and probably *A. maculatus* carrying malaria, *Aëdes albopictus* carrying dengue, *Culex tritaeniorhynchus*, *C. quinquefasciatus*, and *C. pipiens* Japanese B encephalitis. All these species except *Aë. albopictus*, *A. minimus*, and *A. maculatus* may carry filariasis. Breeding of mosquitoes took place in streams, paddies, artificial containers, and water courses. Routine procedures were adopted to determine the fluctuations of larval populations for evaluation of the degree of effective control. This control was effected by a program which commenced with the residual spraying of buildings, followed in the order given, by treatment of breeding places within the Army areas, treatment of breeding places outside Army areas but nearby, application of dusts to effect an adulticidal barrier, and fogs to give an adult knockdown or a kill of the *pro tempore* adult population.

Other insects, such as flies of the genera *Musca*, *Chrysomyia*, *Lucilia*, *Eusimulium*, *Psychoda*, and *Chironomus* carried dysenteries, or caused discomfort and psychosomatic diseases of various sorts. *Tribolium* affected health by spoiling the bread supply. Various wood borers, pest household insects, and insects attacking shrubbery or shade trees alarmed and distressed the personnel of the Army posts, and came within the scope of the control activities.

Termites were a problem in all areas especially where poor construction caused improper or termite-fostering conditions. Control was obtained by soil poisoning and impregnation of replacement timber with pentachlorophenol and by blowing DDT 10 per cent dust into channels. Rattan and bamboo were damaged by bostrychid beetles which were controlled by saturation spraying with 5 per cent DDT in kerosene. Pine infestation by bark beetles was controlled by spraying with 5 per cent DDT in water suspensions.

Resistance was found among lice in Korea, but no resistance in flies, fleas, or mosquitoes was noted up to the end of 1951. It was concluded that, although the breeding sites available to insects are numerous in the areas concerned, effective general control of disease-bearing insects was feasible.

DR. BENJAMIN GARRISON THOMPSON JOINS POINT FOUR MISSION IN IRAN

Dr. Benjamin Garrison Thompson, economic entomologist, of 1397 Alta Vista Road, Corvallis, Oregon, departed in December 1952 for Iran to join the Point four mission of the technical Cooperation Administration, Department of State. Mrs. Thompson and their daughter Margaret are accompanying him.

Dr. Thompson since 1924 has been doing experimental work in his specialty at the Oregon State College Experiment Station, and is the joint author of some 50 technical papers. Born in Linn County, Oregon, he has B.S. and M.S. degrees from Oregon State College and a Ph.D. degree from the University of Washington, Seattle. He taught school in Linn County, and later served with the Bureau of Entomology of the U. S. Department of Agriculture. Principal Point 4 concerns in Iran are agriculture, natural resources, health and sanitation and education.

The Importance of Some Biological Characteristics of European Corn Borer on Timing of Application of Insecticides[1]

H. C. Chiang and L. K. Cutkomp,[2] *Division of Entomology and Economic Zoology, University Farm, St. Paul, Minn.*

Insecticidal treatment is recommended for the control of the first-generation corn borer in field corn 10 to 12 days after the first eggs have hatched if the number of egg masses in the field reaches 50 or more per 100 plants on any one day during the period of egg deposition. The critical level of infestation indicating a need for insecticidal treatment has been evaluated by Chiang & Hodson (1952) from an economic viewpoint. They suggested that one of the important factors which must be used in the determination of this critical level of infestation is the efficiency of insecticidal control to be expected. In the present paper a theoretical evaluation is made of the efficiency of insecticidal control of this insect with particular reference to the recommended method of timing.

Inasmuch as the importance of spray timing is to be evaluated, it is necessary to be able to distinguish its effect on the efficiency of an operation from effects due to the toxicity and residual properties of an insecticide and the method of application. Under field conditions, it is usually difficult to make this distinction, and at the same time account for the effects of seasonal weather variation and host varietal differences. For the purpose of making an analysis of the timing procedure, some of the complications can be minimized by assuming a much higher degree of insecticide effectiveness than can be expected in practice, thus eliminating from consideration the properties of the insecticide and the method of application. Having made this assumption and having acquired a knowledge of the pertinent biological characteristics of the corn borer, it would then be possible to assess the vulnerability of corn borer larvae to the action of an insecticide applied at various times during the hatching period. In the present study the importance of timing and the possible degree of success in the control of the corn borer that can be expected by the use of insecticides is considered from this theoretical point of view. The theoretical presentation is followed by examples taken from actual field tests which serve to place the calculated estimates of control efficiency in proper perspective.

Most of the data upon which the present analysis is based were obtained from observations made on some biological characteristics of corn borer during four seasons, 1948 to 1951, at the Southeast Experiment Station, University of Minnesota, Waseca, Minnesota.

Varieties, Dates of Planting and Seasonal Conditions.—A total of twelve plantings of corn were under observation. The varieties of corn used and the dates of planting are given in table 1. The hybrids 503, 504 and 505 have a maturity date of 108 days, whereas hybrid 608 matures in 101 days.

The weather conditions at Waseca, Minnesota, during the four growing seasons can be described briefly as follows: the year of 1948 had a hot summer, a late fall, and average precipitation; 1949 had an early season, both summer and fall, and had below average precipitation during the late summer; 1950 had a very late spring, an exceptionally cool summer, a fairly early fall, and adequate precipitation; 1951 was quite similar to 1950, though having even greater precipitation.

The number of borers present at the time of fall surveys in Waseca county during the 4-year period varied a great deal. The average number of overwintering borers per 100 plants in the falls of 1947, 1948, 1949, and 1950 were 437, 99, 1,230 and 70 respectively, according to the reports of the Office of the State Entomologist.

Methods.—Data were gathered on the seasonal progress of egg deposition, the seasonal progress of egg hatching, the distribution of larvae on the plants and

[1] Paper No. 2945 Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul, Minnesota.

[2] Grateful acknowledgment is due to Drs. A. C. Hodson and F. G. Holdaway who offered suggestions during the preparation of the manuscript, and to Mr. R. E. Hodgson, Superintendent of the S. E. Expt. Sta. of the University of Minnesota, who provided field plots and material assistance.

Table 1.—Planting dates and corn varieties used during 1948 to 1951.

Minhybrid Number and Planting Sequence	Year	Planting Date
505-1	1948	May 20
503-1	1949	May 3
503-2	1949	May 17
504-1	1950	May 12
504-2	1950	May 22
504-3	1950	May 31
504-4	1950	June 12
608-2	1950	May 22
608-3	1950	May 31
608-4	1950	June 12
503-3	1951	May 20
503-4[1]	1951	May 23

[1] Corn planted at Madelia, Minnesota.

the larval survival on plants of different heights. The methods employed in securing this information are presented in the following paragraphs.

(1) Egg deposition and egg hatching of the natural infestation. Numbered corn plants in plots were examined approximately twice a week throughout the period of egg laying and hatching of the first generation. These plants were randomly distributed throughout the plots. The numbers of unhatched and hatched eggs were recorded at each inspection.

(2) Larval survival on plants of different heights, using an artificial infestation. Plants of different heights were artificially infested each with 3 egg masses,[3] or about 48 eggs. The number of larvae which survived was checked by dissecting three plants ten days after the eggs hatched. The quantitative relationship between the normal height of the plants at the time of hatching and the survival of the larvae on the plants was used to estimate the survival of the larvae which hatch naturally during different times of the season.

(3) The distribution of larvae on plants using natural infestation. At least 20 plants which were naturally infested with egg masses were dissected about twice a week to check the number and location of the larvae. These dissections were made only on the earliest planting of each variety in each year. The percentage of larvae present in the stalks and on other parts of the plants at each inspection was determined.

BIOLOGICAL CHARACTERISTICS OF THE CORN BORER.—In this section we shall present the results obtained in studying certain biological characteristics of the European corn borer. The relationship of these results to the efficiency of chemical control will be analyzed in another section.

Egg Deposition and Egg Hatching.—Table 2 shows the highest number of egg masses per 100 plants found on any one day during the period of oviposition, the number of eggs laid per 100 plants and pertinent records on egg hatching in the different plantings. Insecticidal treatment would be justified according to the current recommendations for only the four of the twelve plantings that had egg mass counts greater than 50 for any one day.

Our main discussion will be concentrated on these four plantings. However, a comparison of the records on the whole series of plantings brings out some significant indications. Because the efficiency of the insecticidal treatments may be influenced by the length of the hatching period, this aspect is given careful consideration. In plantings of the same year, there are indications of a longer period of hatching in plantings having a larger number of eggs hatched. This was probably due mainly to the differences in the dates of planting. Late plantings received fewer eggs because they did not reach the stage which was suitable for egg laying until the period of egg deposition was at least half over, and consequently hatching on late plantings will take place only during the latter part of the normal hatching period. The length of this hatching period is therefore shorter than on early plantings. A more striking difference in the length of the period of egg hatching will be shown by comparing the plantings of different years. There are general indications of a longer period of egg hatching in years with low average temperature during that period. The contrast is sharply shown between plantings 503-1 (1949) and 608-2 (1950). The numbers of eggs hatched in these two particular plantings were very close, yet the period of egg hatching was almost twice as long in the 1950 planting as in the 1949 planting. Therefore, the results indicate that the temperature effect is quite important in influencing the period of time required for seasonal egg hatching

[3] Sincere thanks are due to Dr. F. G. Holdaway, Division of Entomology, University of Minnesota, who supplied the egg masses used in this study.

but that the planting date may also be quite important indirectly because the condition of the growing corn plant will influence the period over which eggs will be deposited and hatched.

Differential Larval Survival.—By artificially infesting corn plants the survival of larvae on plants of Minhybrid numbers 504 and 608 was shown to be greater at increasing heights (Table 3). The data support findings of Beard & Turner (1942), Luckmann & Decker (1952) and others, namely, that there was a higher rate of larval establishment on the taller plants or plants closer to tasselling.[4] The data also show that the larval survival in relation to the height of the host plant was much greater in Minhybrid 608 than in Minhybrid 504. This difference calls attention to the need for recognizing the importance of plant varieties in this connection. Since the differential larval survival was determined quantitatively only in the two varieties planted in 1950, the analysis of its effect on the efficiency of control as will be presented later will be confined to these two varieties.

Location of Larvae on the Plants.—Table 4 shows the distribution of larvae from natural infestations in stalks and in other parts of the plants on several selected days after first hatching. The results indicate that the rate at which larvae establish in stalks varies considerably from season to season. For instance, during the early part of the 1948 season, a greater percentage of larvae were found in the stalks than at a similar time in 1949; but by the end of the third week following the first hatching, larval establishment in the stalks was similar for the 2 years. In 1950 and 1951 the rate at which larvae became established in stalks was unusually low. The cool weather during these two seasons (particularly during the first 10 days of hatching) was an obvious factor which may reduce this rate of establishment in stalks, but growth of the corn plant undoubtedly played some part.

EFFECT OF THE BIOLOGICAL CHARACTERISTICS ON EFFICIENCY OF INSECTICIDAL CONTROL.—The results of the study of the biology of the corn borer larvae have been presented. Aspects which have some bearing on the efficient use of insecticides have been investigated. To re-

[4] The survival of the larvae is related to the growth of the corn plant. The recent work of Luckmann & Decker emphasizes the tassel ratio as being the best measure of this growth. However, the corn height is also very useful as a measure of plant growth particularly when field conditions are fairly uniform and the comparisons are made within a given variety. We feel justified in using the normal plant height in presenting our data on differential larval survival.

Table 2.—Field records of egg deposition and egg hatching in 12 plantings of field corn naturally infested during 1948 to 1951, in Waseca, Minnesota.

Planting No.	Year	No. Plants Checked	Highest No. Egg Masses per 100 Plants on One Day	Total Eggs Deposited on 100 Plants During the Season	Total Eggs Hatched per 100 Plants During the Season	Date First Hatching	Days of Hatching Period	Av. Temp. of Hatching Period (° F.)
505-1	1948	400	12	381	325	July 5	10	79
503-1	1949	90	87[1]	2,782	1,631	June 20	18	75
503-2		90	27	889	483	June 20	14	74
504-1	1950	40	177[1]	5,798	2,973	June 26	36	68
2		40	90[1]	3,533	1,833	June 26	38	68
3		40	12	532	265	July 11	22	68
4		40	15	175	110	July 21	9	67
608-2	1950	40	83[1]	2,953	1,780	July 1	32	69
3		40	32	871	562	July 5	27	69
4		40	10	120	17	July 21	9	67
503-3	1951	48	47	1,754	925	July 5	25	70
4[2]		48	25	1,057	468	June 28	31	70

[1] Plantings with egg mass counts over 50. The borer infestation in these plantings was heavy enough to have justified insecticidal treatment according to the current recommendations.
[2] Corn planted in Madelia, Minnesota, 50 miles west of Waseca.

Table 3.—Differential survival of larvae 10 days after hatching on plants of different heights. Plants were artificially infested by placing three egg masses (48 eggs) in the whorl. Data on the two varieties planted in 1950.

Average Normal Height of Plants at Egg Hatching	Days Between Hatch and 50% Tasselling	Survivors (from 144 Eggs)	Per Cent Survival
Minhybrid No. 504			
29.5	27	32	22.2
42.8	28	42	29.1
49.3	13	41	28.5
53.4	13	44	30.6
Minhybrid No. 608			
17.4	39	5	3.5
26.8	30	26	18.1
40.9	21	27	18.8
51.2	13	83	57.6

Table 4.—Distribution of larvae in stalks and in other parts of the plants on several days after first hatching. Studies made on natural larval infestations.

Date of Observation	Number of Larvae	Days After First Hatching	Per Cent Larvae in Stalks	Per Cent Larvae in Other Parts of Plant	Cumulative Borer Degree-Days During First 10 Days of Hatching[1]
Planting 505-1, 1948					276
July 16	199	11	15.3	84.7	
19	172	14	17.3	82.7	
24	137	19	40.6	59.4	
Planting 503-1, 1949					239
July 1	108	11	0.9	99.1	
7	188	17	10.1	89.9	
13	291	23	52.9	47.1	
Planting 504-1, 1950					155.5
July 7	66	11	0	100	
12	47	18	0	100	
22	45	26	4.4	95.6	
Planting 503-3, 1951					199.5
July 13	35	8	0	100	
17	61	12	1.6	98.4	
21	92	16	1.1	98.9	
25	108	20	2.2	97.8	
29	119	24	12.6	87.4	

[1] Cumulative total of the average daily temperature in degrees over and above 50° F.

late them to the use of insecticides it is first desirable to consider "perfect insecticidal effectiveness." The assumption is made, therefore, that:

The insecticide after being applied will be present on all important parts of the plants but not *in* the stalks.

The insecticide is capable of killing all vulnerable borers upon contact during its effective period.

The insecticide is effective for 8 consecutive days after it is applied on the plants.

One application of the insecticide is made.

This assumed insecticidal effectiveness is compared with results from actual field tests later in the paper. From the above assumptions it follows that all larvae that hatch before the application of the insecticide will be killed except those that had previously entered the stalks, as will all larvae hatched during the 8-day period following the application of the insecticide. Since the recommended procedure calls for the application of the insecticide 10 days after the first eggs have hatched, the above situation will be equivalent to a complete elimination of the larvae that have hatched during the first 18 days, except for those which entered the stalks during the first 10 days. This assumed condition may not appear

Table 5.—The period of hatching and the per cent control in natural infestations by an assumed effective insecticidal treatment in four plantings of corn.

Planting Number	Year	First Hatching		Date of Recommended Insecticidal Application (Tenth Day After First Hatching)	Date of Cessation of Insecticidal Effectiveness (18 Days After First Hatching)		
		Date	Number of Days Before 50 Per Cent Tassel Appearance		Date	Per Cent Total Hatching	Per Cent Which Would Escape Insecticide
504-1	1950	June 26	29	July 6	July 14	72.3	27.7
504-2	1950	June 26	29	July 6	July 14	76.0	24.0
608-2	1950	July 1	24	July 11	July 19	74.4	25.6
503-1	1949	June 20	22	June 30	July 8	100	0

realistic. But by assuming this perfect effectiveness of the insecticide we can evaluate the possibilities and limitations of the factor of timing alone.

In the following paragraphs, we shall discuss the effect of certain biological characteristics on the efficiency of control.

The Egg Hatching.—By the assumption of a perfect effectiveness of the insecticide, the efficiency of the control will then be governed by the relative numbers of borers that may be contacted by the insecticide. Those larvae which escape the insecticide are (1) those that have entered the stalks before the insecticide was applied (first 10 days), and (2) those that hatch after the eighteenth day. At the present moment, however, we shall consider only the effect of a late hatch.

The percentage reduction of the borer population as affected by the factor of egg hatching will be computed from the entire population which hatched during the first 18 days. Table 5 shows the percentage of larvae hatched during the first 18 days of the period of hatching for the four plantings on which insecticidal treatment was justified. The plantings are arranged according to the number of eggs hatched. It can be seen that in a cool season such as 1950 the control estimated on this basis would be about 75 per cent and whereas in a warm season (1949) it would be 100 per cent. This means that for infestations which need to be treated by an insecticide, there may be as many as 25 per cent of the larvae that would not come into contact with the insecticide with the present method of timing if the season is cool. This fraction of the larval population will not be killed by the insecticide no matter how effective the insecticide may be in killing the larvae upon contact.

The Differential Larval Survival.—In our analysis of the effectiveness of the insecticide we have not been considering those borers which would die from causes other than insecticide. Since this "natural" mortality is greater on smaller plants (Beard & Turner, 1942 and previous discussion) the time of application of the insecticide needs to be considered for comparison. One can estimate the expected "natural" mortality by referring to data given in table 4 which indicate the percentage of borers surviving on plants of different heights. By determining (1) the heights of the plants on the first, eleventh and nineteenth days after the first hatch[5] and (2) interpolating the borer survival expected on these days for each of the two varieties (Table 4) one can calculate the number of living or surviving borers which would cause plant injury if no insecticide were applied. These calculated data are presented in table 6, and in table 7 the effect of the insecticide on all borers expected to survive is given. In table 7 the percentage of borers hatching and expected to survive after the eighteenth day will not be subject to control by the insecticide. Thus, for plantings 504-1, 504-2 and

[5] The first to tenth day refers to the period before insecticide application, the eleventh to eighteenth day to the period of insecticidal effectiveness, and the ninteenth day and beyond to the period when no insecticidal effect would be apparent.

Table 6.—Number of larvae expected to survive natural mortality during the three intervals, derived from the number of larvae hatched under natural conditions, and the percentage of larval survival determined by artificial infestation. Data for the two varieties planted in 1950 only.

Planting Number	Interval in Days	Date	Normal Plant Height (in Inches) at Beginning of the Interval	Calculated Per Cent Larval Survival	Larvae Hatched During the Interval	Larvae Expected to Survive the Natural Mortality During the Interval
504-1	1 to 10	June 26–July 5	28.3	22.2	838	186
	11 to 18	July 6–13	46.0	28.0	1311	367
	19—	July 14—	51.8	30.6	824	252
504-2	1 to 10	June 26–July 5	31.3	23.2	904	210
	11 to 18	July 6–13	47.5	28.5	489	139
	19—	July 14—	60.0	30.6	440	134
608-2	1 to 10	July 1–10	28.3	12.2	749	91
	11 to 18	July 11–18	40.3	31.0	573	177
	19—	July 19—	54.2	57.6	458	264

608-2 the percentage control would be expected to be 68.7, 72.3 and 50.4 per cent respectively for these plantings.

The above calculations, based upon the known fact that larvae do not survive in a uniform manner on plants of different growth stages (differential larval survival) was carried out to show more precisely what the insecticide effect could be. If one does not consider the differential larval survival and bases his calculations on eggs hatched and subsequent larval survival (as is commonly done), he would obtain somewhat higher control values of 72.3, 76.0 and 74.4 per cent for plantings 504-1, 504-2 and 608-2 respectively (Table 5). The discrepancy in values is due primarily to the fact that larvae hatching out comparatively late (more than 18 days after the first hatch in the field) would survive considerably better than the average survival rate for larvae throughout the season would indicate. Prolonged hatching, resulting in such a situation, occurred in the cool seasons of 1950 and 1951 and usually may not be a problem in warm seasons.

Location of Larvae on the Plants.—In the preceding paragraphs we have discussed the effect of two biological factors on the efficiency of control: the prolonged period of egg hatching due to cool weather, and the marked differences in natural survival of larvae hatching at different times in the early summer. An additional factor needs to be considered. Those larvae which had entered the stalks before the application of an insecticide would be likely to escape insecticidal action. Thus, the extent of larval establishment in the stalks during the first ten days of larval hatching would also affect the efficiency of control.

Table 7.—The effective control for three of the 1950 plantings.

Planting Number	Larvae Expected to Survive Natural Mortality from Entire Egg Deposition	Larvae Expected to Survive from Eggs Deposited During First 18 Days	Effective Control
504-1	805	553	68.7%
504-2	483	349	72.3
608-2	532	268	50.4%

Referring back to table 3, the percentages of larvae found in the stalks on a certain number of days after hatching were shown for four representative plantings. From that table it can be seen that the percentage of larvae established in stalks within the first 10 days of the hatching period was negligible in 1949, 1950 and 1951, but was a significant fraction (15.3 per cent) in 1948. The high establishment of borers in stalks in 1948 was presumably due to the fact that the weather was unusually warm during the first 10 days of the hatching period. The characteristics of the borer suggests that in a normal season, the numbers established in the stalks probably would not influence the efficiency of the insecticidal treatment applied at the recommended time. However, if it is warm or hot during the early part of the hatching period the insecticidal treatment applied at the recommended time may miss many borers which have penetrated the stalks.

Thus, a warm season may shorten the period of egg hatching but at the same time may increase the chance for the establishment of larvae in the stalks. Considerable larval establishment in the stalks may result from relatively few days of warm weather during the early period of hatching, when early larval establishment in the stalks would not occur during a cool season. It becomes apparent then that very good control with one application will not be probable when the season is either very warm or very cool. The calculated efficiency of control due to the effect of the three biological characteristics of the corn borer is shown for the 12 plantings under observation in table 8.

In the first four plantings for which insecticidal treatment would have been justified, a good control would have been expected only in the 1949 planting (503-1). For the three 1950 plantings, the efficiency of control would have been approximately 50 to 70 per cent depending upon the variety of corn. Special attention should be given to the 1948 planting (505-1). Although the efficiency of control based upon the period of egg hatching would have been 100 per cent, the highest control possible was only about 85 per cent because of the effect of extensive establishment of larvae in the stalks during the first 10 days of hatching as a result of warm weather during that period.

Table 8.—Efficiency of control calculated with consideration of (A) the period of hatching (B) the differential larval mortality and (C) the larval establishment in stalks.

Planting Number	Efficiency of Control with Consideration of		
	A	A and B	A, B, and C
504-1 (1950)[2]	72.3	68.7	68.7
504-2 (1950)[2]	76.0	72.3	72.3
608-2 (1950)[2]	74.4	50.4	50.4
503-1 (1949)[2]	100	(100)[1]	99.1
503-3 (1951)	92	(92)	90.4
608-3 (1950)	91.7	80.3	79.4
503-2 (1949)	100	(100)	100
503-4 (1951)	65	(65)	63.4
504-3 (1950)	91.1	90.1	90.1
505-1 (1948)	100	(100)	84.7
504-4 (1950)	100	100	100
608-4 (1950)	100	100	100

[1] Values in parentheses are estimated. Calculation is not possible because no determination was made on the quantitative relationship between plant height and larval survival.
[2] Egg mass counts sufficiently high to justify treatment.

Table 9.—Calculated expectancy of larval mortality when an insecticide is applied at various times after the first hatching.

No. of Days After First Hatching When Insecticide Was Applied	Per Cent of Total Larval Hatch at the Time of Application	Per Cent of Larvae Hatching During the 8-Day Period Following Application	Per Cent of Larvae Expected to be Killed	Per Cent of Larval Hatching After the 8-Day Period of Insecticide Residual Effect
a	b	c	d	e
Planting 504-1 (1950)				
6	10	47	57	43
8	20	48	68	32
10[1]	28	44	72	28
12	45	37	82	18
14	57	28	85	15
16	68	20	88	12
Planting 503-1 (1949)				
6	43	55	98	2
8	70	30	100	0
10[1]	78	21	100	0
12	91	9	100	0
14	98	2	100	0
16	100	0	100	0

[1] Standard time for insecticide application.

Efficiency of Control with Early and Late Application of Insecticide.—The previous analysis has suggested that the present method of timing may be least effective in very warm and very cool seasons. For this reason it is worthwhile to determine how the efficiency would vary if the insecticide were applied a few days earlier or a few days later than the standard time. We have applied the method of analysis used in the preceding pages to calculate the per cent of the total number of larvae that would be killed by an insecticide applied on the sixth, eighth, twelfth, fourteenth and sixteenth days after first hatching. The calculations were carried out for the plantings in which the greatest numbers of borers hatched in both 1949 and 1950, namely, the plantings 503-1 and 504-1. The results are shown in table 8, with the efficiency of the treatment applied on the tenth day included for comparison.

The percentage of larvae which would have hatched prior to the time the insecticide was applied (Table 9, column b) becomes greater as the application is delayed. In contrast, the percentage of larvae hatching during the 8-day period when the insecticide was assumed to be effective (Table 9, column c) becomes smaller with a delay in application. In other words, an early application would be expected to kill relatively more of the borers which hatch during the effective or residual life of the insecticide, while a late application would kill more of the larvae already on the plants at the time of application. In addition, it is fairly definite that a late insecticide application will kill more larvae during the latter part of the hatching period because its effectiveness would extend a few days beyond that of an application made at the recommended time. A late application, however, would miss more of the larvae which hatched early and could escape by establishment in the stalks.

Seasonal conditions will greatly influence the proper time for greatest insecticidal efficiency. For example, an examination of table 4 will show that, in 1948, even with an application made at the recommended time (the tenth day after the first hatching), about 15 per cent of the borers had entered the stalks at the time recommended for insecticide application and would have escaped. Any delay in application would have resulted in a higher escape, although it would have caused a higher kill among the borers which hatched during the latter part of the hatching period. On the other hand, in 1950, an unusually cool season, there was no larval establishment in stalks as late as the eighteenth day after the first hatching. In this case, an application as late as the eighteenth day would not permit any additional escape of borers due

to their establishment in the stalks, yet it would kill more of the borers hatching during the latter part of the hatching period. Meanwhile, the effectiveness of treatments applied earlier than recommended is likely to be lower (Table 8, column d), because their effectiveness would be lost sooner than for one applied at the standard time. However, we have pointed out previously that at times when the temperature in the early summer is higher than normal and when larval establishment in the stalks starts early (Table 4, 1948 planting), early applications might kill more larvae before they enter the stalks than applications at the standard time. In such a case more borers would escape insecticidal action during the latter part of the hatching period.

In brief, the relative advantages and disadvantages of early and late insecticide applications depend, to a great extent, on the weather conditions and the degree of borer infestation.

FIELD EVALUATION OF THE ASSUMED INSECTICIDE EFFECTIVENESS.—In the preceding discussion we have shown that even with an assumed ideal insecticide effectiveness, the maximum control to be expected from a single application for control of the borers of the first generation could be as low as 50 to 70 per cent if the initial infestation were high. This comparatively low efficiency of control would be due primarily to certain biological characteristics of the borer.

Up to this point the experimentation has involved only a consideration of biological characteristics of the insect and the assumption of 100 per cent larval mortality for a period of 8 days after the application of the insecticide. Since this high level of efficiency by any insecticide may not be realized in practice, we need to use field data to determine the possible degree of success in the control of corn borer that can be expected by the use of insecticides. Data are drawn from some field insecticide tests on sweet corn. Two experiments can be reported for this purpose.

The first experiment was designed to determine the effectiveness of several insecticidal treatments on border populations of known sizes which were artificially induced. The treatments included DDT emulsion, DDT wettable powder (both used at 1.5 pounds actual insecticide per acre). Ryania wettable powder (6 pounds per acre), Ryania (1.25 pounds per acre) plus sulfox-cide,[1] and parathion wettable powder (0.45 pound per acre), with a water spray as a check. Eight plants (four Golden Cross Bantam and four of a Minnesota Valley hybrid), arranged in randomized blocks in an experimental plot, were sprayed at each treatment. Each plant was artificially infested with 15 egg masses; three masses at 2 days before insecticide treatment, three masses immediately after the treatment and at 2, 4, and 6 days after treatment. The average size of the egg masses was 20 eggs, thus the initial infestation was about 300 eggs per plant. The larval survival on each plant was checked 7 weeks after treatment by dissecting the plants. The survival of borers on watersprayed plants was 3.6 per cent. The survival on the two corn varieties was not sufficiently different to warrant separation, thus the data from the two varieties are treated together. The results are shown in table 10.

The results indicated that the DDT wettable powder was slightly more effective than the DDT emulsion, and that parathion was about equally as effective as Ryania plus sulfox-cide. The highest control obtained in this experiment was 82 per cent with the DDT wettable powder. This level of control exceeds some of the higher mortality values obtained with a natural infestation by Apple & Decker (1950), in spite of the fact that they had a much lower initial infestation (32 egg masses per 100 plants versus 1,500 egg masses per 100 plants in the present study), and more sprays (three sprays versus one spray in the present study). Perhaps the most important reason for this difference is that in the present study the egg masses were concentrated uniformly on the plants within a period of only 10 days, a situation which deviates considerably from what has been shown to be natural at an infestation level of 1,500 egg masses per 100 plants. By reviewing the facts presented earlier regarding the length of the hatching period, one can realize that such a high level of infestation would require egg laying for at least 20 days under natural conditions. Thus, it is likely that the seasonal distribution of egg hatching may influence control efficiency.

Table 10.—Reduction of borer population on plants treated with different insecticides. Plants were infested by introducing 15 egg masses per plant on 8 plants per treatment.

TREATMENT	LBS. PER ACRE	BORERS PER 100 PLANTS AT THE TIME OF DISSECTION	PER CENT REDUCTION OF BORER POPULATION IN EARS	PER CENT REDUCTION OF BORER POPULATION ON WHOLE PLANTS	PER CENT REDUCTION OF INFESTED EARS
Water (check)	—	1337			
DDT, emulsion	1.5	312	90	77	63
DDT, wettable	1.5	237	80	82	75
Ryania, wettable	6	887	28	34	25
Ryania plus sulfox-cide[1]	2.5 0.167	400	70	70	25
Parathion, wettable	0.45	412	85	69	78

[1] N-octylsulfoxide of isosafrole (S. B. Penick and Company, New York).

The second experiment was designed to determine the length of the effective period of a single application of the insecticides. In this experiment, three insecticides were used, a 75 per cent DDT wettable powder at 1.5 pounds per acre, 100 per cent Ryania wettable powder at 6 pounds per acre and parathion wettable powder at 0.45 pound per acre. One hundred plants (Golden Cross Bantam) arranged in randomized blocks in an experimental plot were sprayed with TEPP at 0.4 pound per acre; this procedure had been found valuable in clearing plants of other competing insects, especially predators (Cutkomp & Holdaway, 1950). On the next day, 25 plants were treated with DDT, 25 plants with Ryania, 25 plants with parathion and the remaining 25 plants with water as a check. For each test, five plants were artificially infested, each with three egg masses on 1, 3, 5, 7, or 10 days after the insecticide or water treatment. About 8 weeks after the spraying all plants were dissected to check the surviving borer population. Identical tests were carried out with a Minnesota Valley hybrid. The results are summarized in table 11.

The insecticide parathion was most effective at the beginning, but had a somewhat shorter period of effectiveness, especially when compared with DDT. The effectiveness on the first day and the length of the period of effectiveness of the insecticide varied with the plant variety as much as with the insecticides. Nevertheless, the important fact is that the effectiveness in all cases dropped steadily from about 80 to 100 per cent on the first day down to 10 per cent or less on the eighth day. Thus, at the beginning of the 8-day period the actual effectiveness was very close to the 100 per cent assumed for theoretical analysis, but became considerably poorer toward the end of the the period. Under field conditions, and if the insecticide is applied according to the recommended schedule, egg hatching generally will be more concentrated at the time of treatment and will decrease progressively from that time on. From this one can expect that the actual efficiency of control is likely to be fairly

Table 11.—The per cent mortality of the borer population artificially placed on the plants as egg masses at different times using three insecticides on two sweet corn varieties.

NUMBER OF DAYS AFTER SPRAYING WHEN THE EGG MASSES WERE INTRODUCED	PER CENT MORTALITY OF LARVAE					
	Golden Cross Bantam			Minn. Valley hybrid		
	DDT	Ryania	Parathion	DDT	Ryania	Parathion
1	86	45	100	76	88	88
3	65	5	45	26	11	53
5	18	18	9	33	5	5
7	46	14	11	0	0	0
10	0	0	0	11	0	6
Number of borers per 100 plants in check plots		436			336	

close to the hypothetical case stated before, especially when the infestation level is relatively low.

The rapid drop in the per cent of control within 8 days to only 10 per cent is rather unexpected from what is known about the physical persistence of insecticides on the plants. Parathion for example, may remain as 15 to 20 per cent of the original deposit on fruit after 12 days (Walker 1950). We also have unpublished data indicating the presence of some DDT for 30 days after application to corn plants. This discrepancy can be explained, however, by plant growth and the behavior of the larvae. As the plants grow, the larvae, following the whorl, continuously move toward the newly extended parts of the leaves which have not been contaminated with an insecticide. Therefore, the data cannot be assumed to be a reflection solely of the amount of insecticide present on the plants. Instead, the data imply that the insecticide effectiveness is dependent on an interrelationship between plant growth and larval activity.

In concluding this section, it can be said that, in view of the actual limited effectiveness of insecticides as demonstrated above, the theoretical values of 50 to 70 per cent control at high infestations will seldom be reached in practice. And furthermore, even with insecticides of the same effectiveness and initial corn borer infestations of the same level the efficiency of control still will vary with the variety of the corn plant.

SUMMARY AND CONCLUSIONS.—The efficiency of the procedure of controlling the first-generation corn borer in field corn with the recommended methods of timing is estimated by assuming an ideal insecticide and by taking into account some important biological characteristics of the borers. Three biological characteristics are considered: the progress of egg hatching with respect to the season, the larval survival in relation to the height of the host plants, and the time of larval establishment in the stalks with respect to the season.

The following points are shown by our observations:

1. The seasonal progress of egg hatching is slower in a cool season than in a normal season. Thus, in a cool season some larvae would hatch after the dissipation of the insecticide and would escape contact with the insecticide. As a result, the efficiency of the control which is governed by the relative number of eggs hatching before and after the dissipation of the insecticide would be lower in a cool season than in a normal season. The efficiency of control evaluated on this basis may be as low as 75 per cent.

2. A differential larval survival is shown in relation to the height of the host plants. During the same season, the rate of survival of the larvae increased with the increase in plant height. Thus, larvae that hatched early in the season would have a poorer chance for establishing themselves than those that hatched late in the season. Since an insecticide applied according to the recommended method of timing would kill mostly the larvae that hatched early in the season with the poorer chance for survival, the mortality figures would not truly represent the efficiency of the insecticide. When we eliminate natural mortality, only about 50 to 70 per cent of the remaining larvae would be vulnerable to insecticidal action.

3. The time of larval establishment in the stalks is earlier in a warm season than in a normal season. Thus, in a warm season some larvae would enter the stalks before the application of an insecticide and would escape contact with the insecticide.

4. The efficiency of an insecticidal treatment applied a few days earlier or a few days later than the recommended time was estimated. The relative advantages and disadvantages depend, to a great extent, on the weather conditions.

5. Field tests, using an artificial introduction of egg masses, showed that the initial effectiveness varied with the insecticide and also with the variety of corn. In general, the mortality of borers due to insecticidal treatment dropped from 80 to 100 per cent on the first day of application to about 10 per cent on the eighth day. The loss in effectiveness is not only due to decomposition of the insecticide, but also depends upon the growth of the plant and the behavior of the larvae.

Evidence from this study suggests that consistently good results of control under all weather conditions will not be possible by timing the application of an insecticide by a fixed number of days after the

first hatching, whether 10 days, 12 days or any other number of days. As a result of this analysis one can conclude that a more flexible system of timing is desirable which is guided by biological events which are self-adjustable to changes in weather conditions. In addition, certain of the biological characteristics of the corn borer make it unlikely that improvement in the insecticidal control of this pest is to be found by searching merely for a more toxic material.

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Parthenothrips dracaenae, a Non-Vector of the Spotted Wilt Virus[1]

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Parthenothrips dracaenae (Heeg.), a well-known and injurious greenhouse thrips in Europe and North America, was recently discovered on the island of Oahu (Carter 1953). This species is a possible vector but has never been tested for ability to transmit the spotted wilt virus (*Lethum australiense* H.) which is quite troublesome in Hawaii. So far the virus is known to be transmitted by four different species of thrips elsewhere in the world, and it is probable that more vector species exist. The following transmission tests were conducted during the period from April to October, 1952.

Parthenothrips dracaenae thrived well on *Emilia sonchifolia* DC., a standard source and also test plant of the virus, under experimental conditions. Combined period of egg and larval stages was about 30 to 35 days in these months, the range of monthly average mean temperatures being 73° to 78.5° F. Life cycle from egg to egg was about 50 days. A large number of healthy adults was transferred to caged *Emilia* plants which were stripped of all the leaves except the ones mechanically inoculated with infected plant sap at the time of insect transfer. The earliest systemic symptoms appear on terminal leaves in 5 to 7 days after inoculation and the inoculated leaves also harbor a high concentration of the virus, although local lesions seldom appear. With this procedure, eggs were laid in these inoculated leaves or other leaves formed after inoculation, and offspring larvae which began to hatch in about a week after transfer were all forced to feed on nearly infected leaf tissues which were carrying a high concentration of the virus. Since all the known vector species acquire the virus during their larval stage only, acquisition feeding of the experimental insects was limited to their larval stage. Full grown larvae, prepupae, or pupae which fed on infected tissues during their whole larval period were transferred to healthy *Emilia* plants for test feeding. Such transfers were made 30 to 40 days after the original adults were transferred to the infected plants for acquisition feeding. The test plants were kept under observation for 30 days or more.

In the first test, 360 insects from three different colonies on diseased plants were transferred to 24 test plants confined in 12 cages. In the second test, 180 insects from 3 different colonies were transferred to 36 test plants confined in 4 cages. In the last test, 300 insects from 5 different colonies were transferred to 30 test plants confined in 15 cages. In these three tests, 840 insects in total were tested and none of 90 test plants became infected. Considering the experimental method employed and the number of insects tested, the evidence is sufficient to safely conclude that *Parthenothrips dracaenae* is not a vector of the spotted wilt virus.

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[1] Published with the approval of the Acting Director of the Pineapple Research Institute of Hawaii as Technical Paper No

The Isomerization of Organic Thionophosphate Insecticides[1,2]

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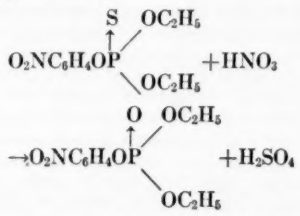
The original studies on the mode of action of parathion in mammals and in insects (Dubois *et al.* 1949, Metcalf & March 1949, Aldridge 1950, Grob 1950, and others) indicated not only that this compound was a strong inhibitor of cholinesterases *in vivo*, but also that it inactivated about 50 per cent of various cholinesterases at concentrations of the order of 10^{-6} M. *in vitro*. More recent work by Diggle & Gage (1951), however, has shown that very highly purified parathion is only a feeble inhibitor of cholinesterases *in vitro*, whereas impure preparations show much higher anticholinesterase action. These authors have also demonstrated that the *in vitro* anticholinesterase action of impure parathion is due to traces of the parathion isomeride, O, S-diethyl O-*p*-nitrophenyl thiophosphate, which is a very active anticholinesterase. It was indicated, therefore, that the earlier work on the *in vitro* inhibition of cholinesterases by parathion was carried out with samples contaminated by traces of this isomeride.

The toxicities of the isomerides of parathion and of methyl parathion (O,O-dimethyl O-*p*-nitrophenyl thionophosphate) to insects and mammals have been studied by several investigators. Martin (1950) records that O,S-diethyl O-*p*-nitrophenyl thiophosphate is about one-fourth as toxic as parathion to *D. oleraceae* as a stomach poison, and about one-sixteenth as toxic to *C. granaria* by contact. In contrast, however, this isomeride was found to be about 10 times as toxic as parathion to the white rat by subcutaneous injection. Schrader (1951) found the S-ethyl isomer of parathion to be less than one-fifth as effective against aphids as parathion, and less than half as toxic to the white mouse by subcutaneous injection. Hecht & Wirth (1950) found S-ethyl parathion to be about 3 times as toxic to the frog as parathion, about half as toxic subcutaneously to the white mouse, and about one-thirteenth as toxic orally to the white rat. The S-phenyl isomer was about 20 times as toxic to the frog, 6 times as toxic to the mouse, and twice as toxic to the rat. Similar results were obtained with methyl parathion and its S-methyl and S-phenyl isomerides. Aldridge & Barnes (1952) reported that, when injected intravenously in male rats, S-ethyl parathion was slightly more than twice as toxic as parathion, while the S-phenyl isomer was 6 times as toxic.

The present investigation was conducted with the hope of clarifying these somewhat divergent findings and assessing the importance of the isomerization phenomenon on the mode of action, decomposition of residues, and insect and mammalian toxicity of the thionophosphate insecticides.

The isomerization of thionophosphates was first discussed by Emmett & Jones (1911), who showed that when O,O,O-trimethyl thionophosphate, $D^{10.5} = 1.2112$, $n_D^{10.5} = 1.45830$, was heated in a sealed tube to 100° C., about 50 per cent was converted to 0,0,S-trimethyl thiophosphate, $D^{10} = 1.2565$, $n_D^{10} = 1.46864$. The maximum conversion was reached after 3 to 4 hours and was never complete. Schrader (1947) referred to the heat isomerization of parathion at 130° to 140° C. and stated that the early products of E-605 contained up to 5 per cent of the S-ethyl isomeride. He noted that the extent of isomerism of thionophosphates, and the consequent conversion of $\equiv P \rightarrow S$ to $\equiv P \rightarrow O$, can be measured by determination of the thiono-sulfur. This can be done conveniently by oxidation with 55 per cent nitric acid, according to the following reaction:



Although most investigators have considered the S-ethyl isomer as an undesirable impurity in parathion, Morill (1952)

[1] This is the third paper of a series concerning the mode of action of organic phosphorus insecticides.

[2] Paper No. 749, University of California Citrus Experiment Station, Riverside, California.

has patented this compound as an insecticide "superior to parathion." He states that it was produced in 54 per cent yield by heating parathion at 140° to 180° C. for 5 hours. Gage (1952) has recently developed a colorimetric technic for determination of S-ethyl parathion.

MATERIALS AND METHODS.—The organic thionophosphate esters used in this study were highly purified to eliminate traces of the various isomerides. The compounds included O,O-diethyl O-*p*-nitrophenyl thionophosphate (parathion),[3] 99.8+ per cent pure, a colorless nearly odorless liquid melting at about 6° C., $n_D^{20} = 1.5384$; O,O-dimethyl O-*p*-nitrophenyl thionophosphate (methyl parathion),[3] m.p. 36° to 37° C., and O,O-diisopropyl O-*p*-nitrophenyl thionophosphate (isopropyl parathion),[3] m.p. 56° to 57° C., both white, odorless solids recrystallized 4 times from ethyl alcohol; O,O-dimethyl S-(1,2-dicarbethoxyethyl) dithiophosphate (malathon),[3] 99+ per cent pure, $n_D^{20} = 1.4991$, a colorless liquid with a faint sulfurous odor; and also ethyl *p*-nitrophenyl thionobenzenephosphonate (EPN),[4] m.p. 34° to 35° C., a white nearly odorless solid, recrystallized 6 times from petroleum ether. Methyl parathion was used in most of the experiments because of the ease of its purification in relatively large amounts and the convenience in handling a solid.

Isomerization of 0.5- to 2-gram samples of these materials was accomplished by heating in sealed tubes to 150° C. for various periods of time. The extent of isomerization was measured by determining the thiono-sulfur content by refluxing a precisely weighed sample for from 3 to 4 hours in 5 ml. of 55 per cent nitric acid. The excess nitric acid was volatilized by heating, and the sulfuric acid produced was determined by precipitation with benzidine and subsequent titration with standard base (Furman 1939). Total sulfur was determined gravimetrically by oxidation in the Parr bomb and precipitation as barium sulfate (Shriner 1941).

The toxicity tests on 2- to 4-day-old female house flies of a susceptible laboratory strain were made by the topical application of measured 1-microliter drops of standard acetone solutions, and the oral toxicities to white mice were determined by the administration of measured amounts of propylene glycol solutions. The 24-hour mortalities were plotted as dosage-mortality curves on log.-probit paper, and the LD_{50} values determined by inspection. The determinations of anticholinesterase activities of the various materials were made by the manometric method, as previously described (Metcalf & March 1950), using as enzyme brei 3 homogenized housefly heads per ml. of buffer solution, or one mouse brain per 20 ml. of buffer. Inhibitors were added in 0.1-ml. aliquots of acetone.

Reversed-phase paper chromatography of the various phosphate and thionophosphate-esters was carried out using Whatman No. 1 paper impregnated with a silicone[5] and the upper phase of a mixture of anhydrous ethyl alcohol (10 parts), chloroform (10 parts), and water (6 parts) (Metcalf & March 1953).

DISCUSSION OF RESULTS.—The data in table 1 show the relative inhibition of fly-brain cholinesterase by samples of purified insecticides, as compared with impure or technical samples. It is apparent that purification in all cases reduced the anticholinesterase activity. Samples of the purified materials were then heated to 150° C. in sealed tubes. Determinations of the total-sulfur and thiono-sulfur of parathion and methyl parathion, and of their heat-produced isomerides, are given in table 2. It will be seen that the heat treatment resulted in the loss of about 80 to 90 per cent of the thiono-sulfur, whereas the total-sulfur content decreased only about 10 per cent. This loss is probably accounted for by the decomposition of a small amount of the starting material, as was indicated by the formation of a highly volatile fraction with a disagreeable-smelling, mercaptan-like odor.

More specific results of such experiments with the various insecticides, and the biological properties of the heat-produced isomerides, are detailed below.

Methyl Parathion.—When the pure crystalline compound was heated for 3.5 hours at 150° C., a faintly yellowish, very viscous liquid was obtained which had a pronounced mercaptan-like odor. Analysis indicated 83 per cent isomerization. Similar results were obtained with another sample heated 6.5 hours, when 91

[3] Obtained through the cooperation of Dr. J. L. Horsfall of the American Cyanamid Company.
[4] Obtained through the cooperation of Dr. S. S. Sharp of the E. I. duPont de Nemours & Co. (Inc.).
[5] Dow Corning 550 Fluid.

per cent isomerization occurred. The isomerized samples were more than 1,000 times as effective as inhibitors of fly-brain cholinesterase, and 80 times as effective against mouse-brain cholinesterase, as the starting material, but were much less toxic to flies and mice (Table 3). Heating at 150° C. for 24 hours or longer resulted in at least partial decomposition, producing two phases: (1) a very volatile, mobile liquid, and (2) a muddy solid.

Parathion.—Purified parathion heated for 24 hours to 150° C. resulted in 80 per cent isomerization in one trial and 94 per cent in a second. The refractive index was changed from $n_D^{20}=1.5384$ to $n_D^{20}=1.5444$, while the sample changed from almost colorless to orange-red. The 94 per cent isomerized sample was about 1,000 times as effective as an inhibitor of fly-brain cholinesterase, and 500 times as effective against mouse-brain cholinesterase, as the starting material, but was less toxic to both flies and mice (Table 3).

Isopropyl Parathion.—Heating of the pure crystalline compound to 150° C. for 3 or 6 hours resulted in the formation of a blackish granular material and a yellowish, very mobile liquid indicative of partial decomposition. A repetition of this experiment with 3 hours' heating at 150° C. resulted in the formation of about one-fourth volume of very mobile liquid and three-fourths volume of brownish, highly viscous material. This latter substance, when separated, was considerably more active against fly-brain cholinesterase than the starting material, yet was much less toxic to the fly (Table 3). When isomerization was attempted at 105° to 110° C. for 6 hours, the original starting material was obtained unchanged.

Malathon.—Heating of the purified, nearly colorless liquid for 24 hours at 150° C. resulted in the formation of an orange-brown, viscous liquid and some colorless cloudy material which was immiscible. The refractive index changed from $n_D^{20}=1.4991$ to $n_D^{20}=1.4982$. The heated material, which was about 90 per cent isomerized, was about 10 times as effective as an inhibitor of fly-brain cholinesterase as the starting material, and was much less toxic to the house fly (Table 3).

EPN.—Purified EPN heated to 150° C. for 24 hours resulted in a dark-brown liquid having a characteristic mercaptan-like odor. This material was 20 per cent isomerized and was about 35 times as effective as an inhibitor of fly-brain cholinesterase and about 60 times as effective against mouse-brain cholinesterase as the starting material, but was slightly less toxic to the house fly (Table 3). Lack of sufficient quantities of material prevented assessing its toxicity to the mouse.

O,O-diethyl O-2-ethylmercaptoethyl thiophosphate.[6]—A technical sample of this material contained 52 per cent of the theoretical amount of thiono-sulfur, indicating the presence of nearly equal amounts of the two isomerides: O,O-diethyl O-2-ethylmercaptoethyl thionophosphate and O,O-diethyl S-2-ethyl-mercaptoethyl thiophosphate.

Isomerization as Measured by Cholinesterase Inhibition.—Since the isomerization of the thionophosphates has been shown to result in products which exhibit a greatly increased effectiveness as anticholinesterases (Table 1), it follows that by plotting a standard curve of concentration vs. percentage of cholinesterase inhibition, for the nearly pure isomeride, one may use this reaction as a measure of extremely small amounts of isomerization. Such a curve for isomerized methyl parathion (91 per cent) is shown as figure 1. It will be seen that concentrations of isomeride as small as 10^{-7} M. can readily be detected in the presence of more than 1000 times as much purified O,O-dimethyl O-*p*-nitrophenyl thionophosphate. Diggle & Gage (1951) have used this method to measure the extent of isomerization of technical samples of parathion; we have found it very useful in following the course of isomerization of purified thionophosphates.

In order to study the isomerization of methyl parathion more completely, a solution of 10^{-2} M. purified compound was prepared in 95 per cent ethyl alcohol. This solution was kept in the dark and refrigerated. Two-ml. aliquots were sealed in 0.5-inch O.D. pyrex tubes for incubation in an oil bath at elevated temperatures or exposure to ultraviolet irradiation from a Hanovia mercury vapor lamp at 22-cm. distance and 19° C. Under these conditions, the results presented in table 4 were obtained. At 50° C. no detectable isomerization in alcohol solution took place

[6] Active ingredient of Systox, obtained through the cooperation of Mr. R. J. Geary of Plant Products, Inc.

in 4 hours, but at 100° C. isomerization occurred readily, the amount of isomeride formed being directly proportional to the time of heating. Similar results were obtained with ultraviolet irradiation, although the amounts of isomeride formed were very much less. Methyl parathion isomerizes very readily, and this reaction will even occur upon long standing on the laboratory shelf. For example, a 1×10^{-2} M. solution of this compound in ethyl alcohol, when freshly prepared, produced 5 per cent inhibition of fly ChE at a dilution of 3.3×10^{-5} M. After standing for 5 months, the same solution gave an inhibition of 94 per cent at this concentration.

Hydrolysis of Isomerides.—The fact that the greatly enhanced *in vitro* anticholinesterase activity of the thionophosphate isomerides does not result in high *in vivo* toxicity, but is, in fact, associated with a large reduction in toxicity, is puzzling. The most logical explanation seems to be that the isomerides are so unstable to hydrolysis that they are largely destroyed before reaching the site of action, unless injected intravenously, when they have been reported as being relatively more toxic (Aldridge & Barnes 1952). To test this hypothesis, the alkaline hydrolysis of these materials was compared at 37° C. in standard sodium hydroxide solutions, using the rate of formation of *p*-nitrophenate ion, as measured spectrophotometrically by absorption at 400 mμ. A Beckman spectrophotometer with constant-temperature absorption-cell chamber was used in these experiments. The materials were prepared at 10^{-3} M. in acetone, and 1 ml. of this solution was added to 99 ml. of standard alkali at zero time to give a final phosphate ester concentration of 10^{-5} M. Under these conditions, purified parathion was hydrolyzed to produce the theoretical amount of *p*-nitrophenate ion. Hydrolysis values were calculated from the true second-order rate equation $K_{hyd}=(2.303/t)\log_{10}(a/b)[OH^-]^{min^{-1}}$, where t = time in minutes, and a = the original and b = the final concentration of ester, respectively; and the values presented in table 5 were obtained. On the assumption that the S-alkyl isomeride was the principal product of the heat isomerized parathion, it appears that the isomeride hydrolyzes nearly 300 times as fast as parathion and nearly 40 times as fast as para-oxon.

With the isomeride of methyl parathion, the results obtained were less clear-cut. The initial hydrolysis in 0.001 *N* NaOH was very rapid until about 20 per cent of the theoretical amount of *p*-nitrophenate was formed, in 10 minutes, when hydrolysis almost completely ceased. Even in 0.1 *N* NaOH, a maximum of about 27 per cent of the theoretical *p*-nitrophenate was formed in 24 hours.

Paper Chromatographic Separation of Isomerides.—Reversed-phase paper chromatography provided an excellent means for separation and identification of the normal constituents of technical parathion and methyl parathion, as well as those of the heated products.

When several samples of technical parathion from different manufacturers were chromatographed, as previously described, three yellow spots were consistently obtained by development with alcoholic KOH. These had average R_F values of 0.04, 0.47, and 0.78, and were apparently O,O-diethyl O-*p*-nitrophenyl thionophosphate, O,S-diethyl O-*p*-nitrophenyl thiophosphate, and *p*-nitrophenol, in ascending order. This was deduced by comparison with known materials, and by the fact that the upper spot was yellow immediately, without hydrolysis, whereas the middle spot hydrolyzed very rapidly, and the bottom spot only slowly, with heat.

The spots were eluted and quantitatively determined by absorption at 400 mμ, using a Beckman DU spectrophotometer with the results shown in table 6. It will be noted that the proportion of S-ethyl isomer ranges from 5 to 20 per cent; this readily accounts for the high anticholinesterase activity of these samples of technical parathion.

Table 1.—Inhibition of fly-brain cholinesterase by thionophosphates of different purities.

	Sample		IN_{50}[1]
A. Parathion		99.8+% pure	$>3.3\times10^{-4}$
		molecularly distilled	4.5×10^{-7}
B. Methyl parathion		4×recrystallized	$>3.3\times10^{-4}$
		technical crystals	1.3×10^{-5}
C. Isopropyl parathion		4×recrystallized	$>3.3\times10^{-4}$
		technical crystals	2×10^{-5}
D. Malathon		99+% pure	7×10^{-5}
		90% technical	6×10^{-6}
		65% technical	3.5×10^{-7}
E. EPN		6×recrystallized	6×10^{-6}
		technical	3×10^{-7}

[1] Molar concentration producing 50 per cent inhibition of fly-brain cholinesterase.

Table 2.—Sulfur content of parathion, methyl parathion, and their heat isomerized products.

Compound and Isomerization Treatment	Per Cent Isomerized	Per Cent Sulfur		
		Theoretical	Found	
			Total	Thiono-
Parathion, 99.8+% pure	—	11.0	10.8	11.26
Above, heated at 150° C. for 24 hours	90.0	—	9.8	0.99
Methyl parathion, m.p. 36-37° C.	—	12.2	12.2	12.46
Above, heated at 150° C. for 4 hours	84.7	—	11.1	1.86

Table 3.—Relative toxicities of certain thionophosphates and their heat isomerides.

Compound	24-Hour LD_{50} in Mg. per Kg.		IN_{50} for Brain ChE	
	Fly, Topical	Mouse, Oral	Fly	Mouse
1. Methyl parathion (purified)	1.3	100–200	2×10^{-4}	8×10^{-4}
Heat isomeride of 1	>500	>200	5×10^{-7}	1×10^{-5}
2. Parathion (purified)	1.4	24–32	$>3.3\times10^{-4}$	$>3.3\times10^{-4}$
Heat isomeride of 2	14	48–64	1×10^{-7}	6×10^{-7}
3. Isopropyl parathion (purified)	4.8	>200	$>3.3\times10^{-4}$	$>3.3\times10^{-4}$
Heat isomeride of 3	>250	—	2×10^{-5}	—
4. Malathon (purified)	27	—	7×10^{-5}	$>3.3\times10^{-4}$
Heat isomeride of 4	>500	—	6×10^{-6}	$>3.3\times10^{-4}$
5. EPN (purified)	2.0	—	7×10^{-6}	3×10^{-4}
Heat isomeride of 5	2.5	—	2×10^{-7}	5×10^{-6}

The chromatographic procedure was also applied to the separation of the constituents of a sample of purified parathion heated in a sealed tube to 150° C. for 24 hours. Eight distinct spots were formed, of which five were identified with some certainty and quantitatively estimated as shown in table 7. The consistency of the method was indicated by values of 56.6, 54.3, and 61.0 per cent for three replicate determinations of amounts of S-ethyl parathion (Calculated as *p*-nitrophenol). The value of 10 per cent thionosulfur remaining after 24-hour heating of parathion at 150° C. is in good agreement with the results of the chromatographic separation, if we consider that both bis-(*p*-nitrophenyl) ethyl thionophosphate and diethyl *p*-nitrophenyl thionophosphate would react to the nitric acid oxidation.

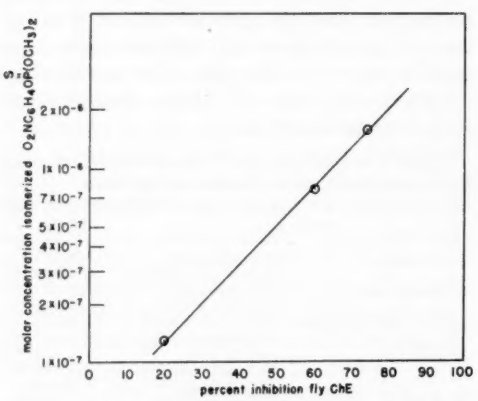


FIG. 1.—Standard curve for relating fly-brain cholinesterase inhibition with molar concentration of heat-isomerized methyl parathion.

Paper chromatography of methyl parathion heated 6 hours at 150° C. resulted in the appearance of four spots after treatment with alkali. The average R_F values were 0.0, 0.25, 0.54, and 0.86. The identity of the two lower spots is uncertain, but the two upper spots were tentatively identified as O,S-dimethyl O-*p*-nitrophenyl thiophosphate and dimethyl *p*-nitrophenyl phosphate, respectively. The isomerized methyl parathion showed evidence of decomposition, in that it was not fully soluble in acetone; therefore, no quantitative estimations were attempted.

SUMMARY AND CONCLUSIONS.—The isomerization of several organic thionophosphate insecticides was investigated by several technics, viz., by measurement of anticholinesterase activity, determination of thiono-sulfur, and by paper chromatography. Parathion and its methyl analogue isomerize readily upon heating to

Table 4.—Isomerization of methyl parathion measured by cholinesterase inhibition.[1]

Reaction Time	Final Molar Concentration for Enzyme Reaction	Per Cent ChE Inhibition	Average Per Cent Isomerization
Incubation Temperature, 100° C.			
0	3.3×10^{-4}	12	0
	3.3×10^{-5}	0	0
30 minutes	3.3×10^{-4}	53	0.2
	3.3×10^{-5}	7	
45 minutes	3.3×10^{-4}	89	1.0
1 hour	3.3×10^{-4}	94	
	3.3×10^{-5}	73	4.5
	3.3×10^{-6}	14	3.0
	3.3×10^{-6}	12	2.1[2]
2 hours	3.3×10^{-5}	90	10.0
	3.3×10^{-6}	39	9.4
	3.3×10^{-6}	30	6.1[2]
3 hours	3.3×10^{-5}	93	
	3.3×10^{-6}	61	25.7
	3.3×10^{-6}	52	17.0[2]
4 hours	3.3×10^{-5}	94	
	3.3×10^{-6}	68	36.4
	3.3×10^{-6}	59	23.6[2]
Incubation Temperature, 50° C.			
4 hours	3.3×10^{-4}	7	0
Ultraviolet Irradiation, 19° C.			
24 hours	3.3×10^{-4}	22	0.4
48 hours	3.3×10^{-4}	33	0.7
72 hours	3.3×10^{-4}	44	1.2

[1] A solution of 10^{-3} M. purified compound in 95 per cent ethyl alcohol heated in sealed tubes.
[2] After standing 2 days in solution.

Table 5.—Hydrolysis rates of 10^{-5} M. phosphate esters at 37° C.

Compound	First-order K Hydrolysis, Min.$^{-1}$ for 1 Molar NaOH
O,O-dimethyl O-*p*-nitrophenyl thionophosphate	0.69
O,S-dimethyl O-*p*-nitrophenyl thiophosphate	—[1]
Dimethyl *p*-nitrophenyl phosphate	7.7
O,O-diethyl O-*p*-nitrophenyl thionophosphate	0.23
O,S-diethyl O-*p*-nitrophenyl thiophosphate	68
Diethyl *p*-nitrophenyl phosphate	1.7

[1] Rate constant not determinable owing to peculiarities of hydrolysis reaction.

Table 6.—Separation of constituents of technical parathion by paper chromatography.

Sample	R_F	Micrograms *p*-nitrophenol[1]	Per Cent Compound
A	0.04	19.5	89
	0.45	1.2	5
	0.78	3.7	6
B	0.04	12.6	75
	0.50	3.4	20
	0.79	1.8	5
C	0.04	18.3	94
	0.47	1.0	5
	0.78	0.5	1

[1] Average of two replicate separations.

Table 7.—Separation of constituents of parathion (heated to 150° C. for 24 hours) by paper chromatography.

R_F[1]	Probable Identity	Micrograms *p*-nitrophenol[1]	Per Cent as PNP
0.01	bis-(*p*-nitrophenyl)ethyl thionophosphate	0.8	2.8
0.04	O,O-diethyl O-*p*-nitrophenyl thionophosphate	1.6	4.6
0.14	?	3.2	8.8
0.25	?	2.1	6.5
0.36	?	2.4	6.6
0.46	O,S-diethyl O-*p*-nitrophenyl thiophosphate	20.6	57.3
0.71	diethyl *p*-nitrophenyl phosphate	3.9	10.9
0.86	*p*-nitrophenol	1.4	3.9

[1] Average of three replicate separations.

150° C., and, at least in the case of methyl parathion, by exposure to ultraviolet irradiation. The resulting products contain only small amounts of thiono-sulfur and have been shown by paper chromatography to consist of a mixture of compounds of which the S-alkyl isomerides are the chief constituents. Malathon and EPN undergo similar heat isomerization at 150° C. Although, when highly purified, the thionophosphates are only feeble cholinesterase inhibitors, the heat-isomerides are very active inhibitors, and the amount of cholinesterase inhibition is a measure of the extent of isomerization. Surprisingly, however, the heat isomerides are much less toxic to the mouse and the house fly than the thionophosphates. Perhaps this may be explainable by the greatly increased susceptibility of the heat isomerides to destructive hydrolysis.

The knowledge of isomerization of the thiophosphate insecticides is important,

since technical parathion may contain from 5 to 20 per cent S-ethyl isomeride, whereas various other technical thionophosphates may contain up to 50 per cent, as in the case of O,O-diethyl O-ethylmercaptoethyl thiophosphate. The presence of the isomerides may give very misleading values as to anticholinesterase activity, hydrolysis rates, and toxicity, and their formation may play a part in the decomposition of residues of these insecticides on plants.

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THE ROCKY MOUNTAIN CONFERENCE OF ENTOMOLOGISTS

TWENTY-FOURTH ANNUAL MEETING
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This is to announce the twenty-fourth Rocky Mountain Conference of Entomologists.

Time: August 23, noon to August 27, noon, 1953.

Place: Colorado A & M College Forestry Summer Camp, Pingree Park, Colorado, 55 miles west of Fort Collins, Colorado.

Nature of Meeting: Very informal, with programs largely of the symposium type with time for discussion and recreation. Arrangements are such that members of the family may enjoy a mountain camp outing while the meetings are in progress.

Expense: The cost will be in the neighborhood of $2.50 per day for adults and $1.25 for children between 5 and 10 years of age. All meals will be served at a central mess hall. The entomologists will be charged a nominal registration fee to cover general expenses and the preparation of a brief report of the meetings.

GEO. M. LIST, *Secretary*

Development and Loss of Insecticide Resistance in the House Fly

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This report is the fifth in a series of studies on house fly resistance. The study of the development and loss of insecticide resistance in the laboratory was designed to guide the planning and interpretation of the field experiments and to provide an indication of further problems in house fly control. Observations made in connection with this study included the rate of development and loss of resistance to various insecticides. The effect on DDT resistance when the DDT-resistant strain of flies were not exposed to DDT but were selected to other insecticides was also studied.

MEASUREMENT OF RESISTANCE.—The test cages were constructed from galvanized wire screen and were 5 inches high and 2.5 inches in diameter. The cages were treated as they passed through the spray cone on a constant-speed treadmill. Since it has been shown that within a given larval population, the degree of resistance is directly proportional to the duration of the larval period, flies used in this test were always reared from larvae representing the complete range of duration of the larval period. (Pimentel *et al.*, 1951). Ten replicates of about 100 flies each for every strain were exposed to each of five concentrations of insecticide for 12 hours. At the end of the 12-hour period all the flies knocked down or dead were removed and the unaffected flies were killed. Both groups were counted and sexed to determine the resistance of each sex. The percentage of mortality of each sex for each concentration was then plotted on log probability paper and concentration necessary to produce a (knockdown) KD-50 estimated.

All the cages were treated and flies placed in treated cages in a separate room. The flies were then placed in a holding room which had a temperature of 80° F. plus or minus 1° F. The nonresistant strain was reared in one room, and the resistant strains were reared in two other rooms. These rooms also had a temperature of 80° F. plus or minus 1° F.

STATISTICAL METHODS.—The data obtained from the experiments were compared statistically using Student's method with argument 't' from Snedecor (1946).

DEVELOPMENT AND LOSS OF INSECTICIDE RESISTANCE.—The development and loss of resistance for DDT resistance was studied and is presented in the first section. In sections following lindane, dieldrin, and parathion resistance studies consider these questions: would DDT-resistant flies develop resistance to another insecticide faster than non-resistant flies; and what effect would the development of another resistance have on the DDT resistance already present.

DDT RESISTANCE.—Strain "A" consisted of flies highly resistant to DDT. These were collected in September, 1949, from a Tompkins county dairy barn which had shown unsatisfactory control when it had been treated the previous June with a spray containing 60 pounds of 50 per cent DDT wettable powder in 100 gallons of water. The flies were brought into the laboratory and inbred for three generations before eggs were obtained for this study. At this time the KD-50 for the males and females respectively was 10 and 28 per cent (Table 1). To increase the level of resistance of this strain, 20,000 flies from each of five successive generations were exposed in DDT treated cages. The cages had a DDT residue of approximately 4,000 mg. per sq. ft. Flies emerging during each generation within a 24-hour period were held an additional 24 hours before exposure in the treated cages. The flies were allowed to remain in the DDT treated cages until 98 per cent were knocked down. The remaining 2 per cent were then used to rear the next generation. By the 5th generation approximately 3 per cent of the flies survived an exposure of 2.5 weeks on a deposit of 4,000 mg. per sq. ft. of DDT. The flies were then allowed to emerge directly into the heavily DDT treated cages. At the tenth generation a 12-hour exposure to the 30 per cent DDT treated test cages knocked down only 2 per cent.

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Table 1.—The level of resistance of several strains of house flies to DDT, lindane, dieldrin, and parathion.

Strain	Generation	Percentage of Toxicant to Produce KD-50 in 12 Hours Exposure							
		DDT		Lindane		Dieldrin		Parathion	
		Male	Female	Male	Female	Male	Female	Male	Female
Non-res.	—	0.018	0.044	0.0051	0.017	0.0074	0.014	0.0026	0.0054
DDT-res. "A"	1	10.0	28.0	0.0068	0.021	—	—	—	—
	5	30.0[a]	30.0[a]	—	—	—	—	—	—
	10	30.0[b]	30.0[b]	—	—	—	—	—	—
	20	30.0[b]	30.0[b]	—	—	—	—	—	—
	26	30.0[b]	30.0[b]	0.064	0.28	0.027	0.049	0.018	0.035
DDT-res. "A_1"	1	30.0[b]	30.0[b]	—	—	—	—	—	—
	5	2.8	7.2	—	—	—	—	—	—
	10	3.0	9.4	—	—	—	—	—	—
	15	0.87	1.5	—	—	—	—	—	—
	20	0.028	0.053	—	—	—	—	—	—
	22	0.018	0.038	—	—	—	—	—	—
DDT-res. "H_1"	1	1.5	4.4	—	—	—	—	—	—
	5	0.15	0.57	—	—	—	—	—	—
	10	0.59	1.3	—	—	—	—	—	—
	15	0.81	0.20	—	—	—	—	—	—
	20	0.018	0.049	—	—	—	—	—	—
DDT-res. "O_1"	1	0.58	2.4	—	—	—	—	—	—
	5	0.17	0.57	—	—	—	—	—	—
	10	0.032	0.081	—	—	—	—	—	—
	12	0.016	0.038	—	—	—	—	—	—
DDT-res. "W_1"	1	1.5	4.5	—	—	—	—	—	—
	5	0.15	0.62	—	—	—	—	—	—
	10	0.024	0.052	—	—	—	—	—	—
	12	0.013	0.041	—	—	—	—	—	—
Lindane-res. "B"	1	10.0	28.0	0.0068	0.021	—	—	—	—
	8	—	—	0.47	0.96	—	—	—	—
	10	10.0	28.0	—	—	—	—	—	—
	17	10.0	28.0	8.8	21.0	—	—	—	—
	26	10.0	28.0	8.8	21.0	—	—	—	—
Lindane-res. "C"	1	0.018	0.044	0.0051	0.017	0.0074	0.014	0.0026	0.0054
	8	—	—	0.62	1.2	—	—	—	—
	17	—	—	11.0	23.0	—	—	—	—
	26	5.2	12.0	11.0	23.0	0.0094	0.037	0.021	0.054
Lindane-res. "C_1"	1	—	—	11.0	23.0	—	—	—	—
	6	—	—	0.33	0.84	—	—	—	—
	10	—	—	0.018	0.055	—	—	—	—
	15	—	—	0.010	0.021	—	—	—	—
	20	—	—	0.0044	0.019	—	—	—	—
Dieldrin-res. "H"	1	1.5	4.4	—	—	0.0067	0.013	—	—
	5	2.5	10.0	—	—	—	—	—	—
	10	4.8	21.0	—	—	0.024	0.041	—	—
Dieldrin-res. "O"	1	0.58	2.4	—	—	0.0077	0.016	—	—
	5	2.9	6.2	—	—	—	—	—	—
	10	2.7	11.0	—	—	0.019	0.036	—	—
Parathion-res. "W"	1	1.5	4.5	—	—	—	—	0.0028	0.0062
	5	12.0	28.0	—	—	—	—	—	—
	10	12.0	30.0	—	—	—	—	0.021	0.054

[a] Three per cent KD for males and 0 per cent KD for females.
[b] Two per cent KD for males and 0 per cent KD for females.

By the twentieth generation, 70 to 80 per cent of this strain survived an exposure of 2 weeks on a deposit of 4,000 mg. per sq. ft. of DDT. No increase in the level of resistance occurred for the next six generations.

As the DDT resistance increased the level of lindane resistance also increased slightly (Table 1). At the twenty-sixth generation DDT-resistant strain "A" was about 3 and 6 times more resistant than the non-resistant strain to dieldrin and parathion respectively.

To ascertain if this highly DDT-resistant strain would lose its resistance, at the twentieth generation 5,000 flies designated as strain "A_1" were reared without further exposure to any insecticide. Three days after the last fly emerged from Strain "A_1", 1000 eggs were collected each day for a period of 5 days. Each 1000 eggs were placed in a rearing jar which contained 4 pounds of standard N.A.I.D.M. medium. After the larvae had pupated, the pupae were then collected from all five jars and placed in one cage. The same procedure was carried on for each successive generation. The level of resistance of this strain was checked periodically.

The data in table 1 indicate the resistance decreased rapidly, but with considerable variation between succeeding generations. The DDT resistance was lost after 22 generations of inbreeding.

LINDANE RESISTANCE.—An experiment was run to find if flies with a low level of DDT resistance would develop lindane resistance at a faster rate than the non-resistant strain. The DDT-resistant flies used were stock split off from the 1st generation of DDT-resistant strain "A" and will hereafter be designated as lindane-resistant "B". The non-resistant flies used were stock split off from the non-resistant strain and will hereafter be designated as lindane-resistant "C". In selecting strains "B" and "C" to lindane 20,000 flies of each strain were reared for each generation. The flies emerging during each generation within a 24-hour period were held an additional 48 hours before exposure in the treated cages. The selection methods were the same as those mentioned previously. For each generation the cages were treated with a concentration of lindane which would produce a KD 98 in 24 hours exposure. The survivors were then used for producing the next generation.

The resistance to lindane for both the DDT-resistant and non-resistant strain increased rapidly. The difference in rate of increase of resistance was not significant between strains "B" and "C" (Table 1).

A group of lindane-resistant strain "C" was split off at the seventeenth generation and will hereafter be designated as lindane-resistant strain "C_1". To find out if this strain would lose its lindane resistance, it was inbred by the same methods as those used with DDT-resistant strain "A_1".

The data in table 1 shows the resistance to lindane decreased rapidly and after the twentieth generation of inbreeding without exposure to any insecticide had lost all of its resistance to lindane.

Note that no decrease in the level of DDT resistance of lindane-resistant strain "B" occurred when it was selected only to lindane (Table 1).

DIELDRIN RESISTANCE.—In this test two DDT-resistant strains were used. The strains were collected from two of the leading dairy counties in different sections of New York State; DDT- resistant strain "H" from Cortland county; and "O" from Delaware county. In each county, approximately 100 flies were taken from each of six widely scattered barns and combined to make a representative strain from the county. After these strains had passed through two generations in the laboratory without treatment in the laboratory, eggs were obtained for the tests.

In selecting these strains to dieldrin 10,000 flies from each strain were reared for each generation. The methods of selection were the same as those used with lindane except a KD-90 was used. Ten per cent of the population surviving each dieldrin exposure were used for producing the succeeding generation.

After 10 generations of selection on dieldrin, Strains "H" and "O" resisted 3 times as much dieldrin as they did at the first generation (Table 1). As the resistance to dieldrin was increasing and while the strains were not being exposed to DDT, the DDT resistance of strains "H" and "O" remained the same or was increasing (Table 1).

As a control a stock of flies was split from each strain at the beginning of the experiment. The flies from strain "H"

were designated as DDT-resistant "H_1" and flies from strain "O" were designated as DDT-resistant "O_1". These two strains were inbred by the same techniques used with DDT-resistant strain "A_1" without further exposure to any insecticide.

As previously found with DDT-resistant strain "A_1", the DDT resistance of DDT-resistant strains "H_1" and "O_1" was lost after a number of generations of inbreeding without exposure to any insecticide (Table 1). The decrease in resistance of DDT-resistant strain "H_1" was variable. The KD-50 for the tenth generation was greater than the KD-50 for the fifth generation of strain "H_1".

PARATHION RESISTANCE.—The DDT-resistant strain, collected from one of the leading dairy counties, Jefferson county, was designated as DDT-resistant strain "W". This strain was collected and reared by the methods mentioned for strains "H" and "O".

In selecting this strain to parathion 20,000 flies were reared for each generation. The method of selection was similar to those used with lindane. The 2 per cent surviving each parathion exposure was used for producing the succeeding generation.

After 10 generations of selection to parathion, parathion-resistant strain "W" was about 9 times as resistant to parathion as it was in the first generation (Table 1). As the resistance to parathion was increasing and while the strain was not being exposed to DDT, the DDT resistance of strain "W" also increased (Table 1).

As a control, a group of flies was split from strain "W" at the beginning of the experiment and designated as DDT-resistant strain "W_1". This strain was inbred by the techniques used with DDT-resistant strain "A_1" without further exposure to any insecticide. As previously found with DDT-resistant strains "A_1", "H_1" and "O_1", DDT-resistant strain "W_1" lost its DDT resistance after 12 generations of inbreedng without exposure to any insecticide (Table 1).

DISCUSSION AND SUMMARY.—The rate of development of insecticide resistance depended upon the degree of selection. The indications are that the rate of development of insecticide resistance also is related to the particular insecticide involved. With DDT and lindane the build-up of resistance proceeded rapidly while with the parathion the build-up of resistance was markedly slower. While flies selected for dieldrin resistance were not selected as rigidly as the flies selected for DDT, lindane, and parathion resistance, the indications are that the rate of dieldrin resistance was within the range of that of DDT, lindane, and parathion. There also appeared to be a limit to the degree to which a strain can resist a particular insecticide.

No significant difference was found between a DDT-resistant and a non-resistant strain in the rate of development of a high degree of lindane resistance. It might be pointed out that the DDT-resistant strain was 50 times more resistant to DDT than the non-resistant strain. On this basis it seems reasonable to assume that a highly DDT-resistant strain, which does show some resistance to other unrelated organic insecticides, would accordingly become resistant to lindane and other insecticides in fewer generations than the non-resistant strain.

When DDT-resistant flies were selected to lindane, dieldrin, or parathion the DDT-resistance did not decrease and in most cases actually increased. That is, when DDT-resistant flies were exposed and selected to another insecticide their resistance to DDT did not decrease while their resistance to the new material increased. If this occurs in the field, the prospect of controlling resistant flies by mere rotation is not a bright one.

Regardless of level of resistance, strains not exposed to DDT over a period of generations lost their resistance. The number of generations required to accomplish complete loss of resistance ranged from 10 to 20, depending on the initial level of resistance. A similar trend occurred with lindane-resistant flies when they were not exposed to any insecticide.

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House Fly Control in New York State Dairy Barns

Wm. J. Goodwin and H. H. Schwardt

The house fly, *Musca domestica* L., remains one of the most difficult of all economically important insects to control. At least by 1948 it was apparent that this insect was becoming so resistant to DDT that DDT was unsatisfactory. As a result of the fly resistance to DDT, lindane was the recommended insecticide in 1949, but within the year flies were resistant to this compound also. Various other insecticides have been used as residual sprays, but flies have developed resistance to all that were not too toxic to be used in dairy barns.

The house fly control program in New York has been complicated further by the ability of the flies to develop slowly throughout the winter in the dairy barns (Matthysee 1951). This winter breeding results in a quick build-up of flies in the spring when the weather becomes mild. Usually the flies are sufficiently numerous to require control by June 15. This date may vary as much as 2 weeks depending on the climatic conditions.

Besides being an important vector of human and animal diseases the house fly causes considerable annoyance to the dairy animal. The dairy industry is the source of over 50 per cent of the farm income in New York State, and a fly control program therefore is essential.

The object of this study was to test as many compounds as possible under barn conditions, in order to determine their effectiveness for fly control, and to study the rate at which the house flies increased their resistance to these various compounds.

In this experiment four methods of application were used to apply nine compounds. One hundred and seventy-two dairy barns were sprayed during the 2-year period (1951 and 52).

Materials, Equipment, and Procedures.—The materials used were 24 per cent lindane wettable powder, 25 per cent lindane emulsion, 50 per cent DDT wettable powder, 24 per cent dieldrin wettable powder, 18.5 per cent dieldrin emulsion, 50 per cent methoxychlor plus 3 per cent lindane,[1] 25 per cent dilan wettable powder, DDT wettable powder with K 3936,[2] and lindane emulsion with Re 1901.[3] The concentrations used in these tests are shown in table 1 as pounds of active ingredients per 100 gallons of water.

All residual spray treatments were applied with a hydraulic sprayer at 350 lbs. pressure. The spray rod used was described by Pimentel (1951),

The barns treated were selected at random in Tompkins & Cortland counties. The treatments were randomized so that any given treatment occurred only once in the same locality, and in addition barns were selected so that in each treatment there were barns with good and with poor sanitation.

The degree of control was determined by the spot counting method developed by Pimentel (1950), in which all the house flies were counted on eleven 2-square foot areas in the barn. The total number of flies on these areas was used as the criterion of control.

The counts were grouped into the following categories: (1) 0–20 flies—excellent, (2) 20–90 flies—satisfactory, and (3) more than 90 flies—unsatisfactory. Both excellent and satisfactory controls were considered practical control of house flies in dairy barns.

The counts were made 48 hours after the insecticides were applied and at intervals of one week thereafter until the count was unsatisfactory for two consecutive weeks.

Methods of Application and Results.—The overall, spot, and strip methods were employed in 1951 and repeated in 1952. The vaporizing method was used in 1951 only.

Overall.—This is the standard residual spray method most commonly used in dairy barns. The entire inside barn surface except the floor is sprayed, applying 1 gallon of spray on 300 square feet or until the point of run-off is reached. A total of 92 barns were treated using six compounds during the 2-year period. The materials, concentrations, and results are shown in table 1. Four of these toxicants provided more than 4 weeks of control in 1951

[1] DuPont Dairy Barn Spray.
[2] Dow Chemical Company.
[3] California Spray-Chemical Corporation.

which was considered effective control. In 1952 effective control was not obtained with any one of the compounds. The control period was only half as long in most cases as that obtained in 1951, indicating the rapid development of fly resistance to these compounds.

Spot Treatment.—This method gave promising results with lindane and dieldrin in 1950 (Pimentel 1951). Briefly it consists of spraying the center part of the barn ceiling, the areas around the calf pens, and around the barn entrances. Approximately one-third as much spray is required for this method as for the overall, and the compounds used therefore were three times as concentrated. The primary precaution was to apply the insecticides where they could not come in contact with the food and water supply of the dairy cattle.

In studying the spot method lindane and dieldrin wettable powders were the materials used in 1951 and 52 with a total of 49 barns being treated.

The spot treatment method has several advantages over the overall: (1) It theoretically requires longer for flies to become resistant since they are initially exposed to relatively high concentrations of toxicant, (2) Less labor, time and material are required, and (3) Since spray is applied only to areas from which it cannot fall into mangers or fountains the milk residue hazard is probably reduced or eliminated.

The materials, concentrations, and results are shown in table 1. In 1951 both lindane and dieldrin gave effective control but by 1952 a very high level of resistance had developed and neither of these compounds gave effective control in New York.

Strip Method.—This method was described by Pimentel (1951). Briefly it involves the cutting of screen wire into inch-wide strips, dipping them in concentrated insecticide emulsions, allowing the strips to dry, and hanging them up under dairy barn ceilings at one foot intervals. This method was enlarged upon by the authors in 1951 and '52. Pimentel (1951) found dieldrin to be the most effective toxicant, and all the strips used during this experiment were dipped in this material. The screen wire was cut into 1 to 2 inch strips, dipped in 18.5 per cent dieldrin emulsion, allowed to dry for 24 hours, and hung in the barns at 2 to 3 foot intervals. The strips were run lengthwise of the barns and tacked to the walls about 6 inches below the ceiling with three-eighths inch flat head tacks. A magnetic hammer was used to facilitate the tacking of the strips to the wall.

Due to the cost of screen wire, as well as the difficulty encountered in cutting and handling it, substitutes were sought. Cloth was the first choice and in 1951 strips of mosquito netting were used, being handled like the screen strips. The mosquito netting had a tendency to sag and curl, so crinoline (a sized meshed fabric) was substituted in 1952. This was found to be far superior in many ways.

House flies appear to be strongly attracted to string hanging in the dairy barn as light cords are usually black with fly specks. This encouraged the authors to employ string strips for house fly control. Balls of No. 30 white twine were dipped in 18.5 per cent dieldrin emulsion and handled like the screen and cloth strips, except that the string strips were placed only 1 foot apart.

A total of 31 barns was treated with various strip methods, and the results are shown in table 1. Five of the original barns treated by Pimentel in 1950 were retreated in 1951 and '52, to determine if house flies could develop resistance to dieldrin at the concentration used on the treated strips. There was no indication of resistance the second year, but in barns treated the third year only 4.4 weeks of control was obtained as compared to 13.0 weeks in the second year. This suggests the house fly will develop resistance to the dieldrin screen strips after two seasons under New York conditions.

Lindane Vaporizing Method.—Two vaporizers were obtained and placed in one dairy barn which was selected as average in respect to sanitation, fly population and air circulation. Each unit was designed to control insects in 15,000 cubic feet of space. The barn used in this experiment had a volume of 28,000 cubic feet. The units used were like those described by Moore (1951). They were maintained at 110° F. and dispersed lindane vapors at the rate of one gram per day per unit.

Excellent control was never obtained and the results were barely satisfactory for 6 weeks during the early part of the fly control season. As the fly population increased the control became unsatis-

Table 1.—Average number weeks of excellent or satisfactory house fly control in New York State dairy barns. 1951 & 1952.

No. Barns		Treatment		Dosage[1]	Ave. Weeks Control	
1951	1952	Method	Material		1951	1952
15	11	Overall	Lindane	2	2.8	1.8
11	—	Overall	Lindane with Re 1901[2]	2	5.1	—
7	8	Overall	DDT[3]	30	8.0	2.9
5	5	Overall	DDT with K 3936[5]	20		
				4	6.0	1.4
10	16	Overall	Methoxychlor & lindane[4]	32	4.2	2.2
—	4	Overall	Dilan	3	—	1.8
7	7	Spot	Lindane	6	6.8	2.3
16	15	Spot	Dieldrin	6	9.8	2.3
—	4	Spot	Dieldrin	4	—	4.0
7	10	Strip[5]	Dield. Screen		13.0	5.1
2	2	Strip[5]	Dield. Cloth		5.0[6]	5.0[6]
3	7	Strip[5]	Dield. String		6.4[6]	8.1

[1] Pounds of active ingredients per 100 gallons of water.
[2] California Spray-Chemical Corporation.
[3] Dow Chemical Company.
[4] DuPont Dairy Barn Spray.
[5] All strips dipped in 18.5% dieldrin emulsion.
[6] Season ended before method became unsatisfactory.

factory and remained so for the remaining 7 weeks of the season.

Discussion.—The overall method using the chlorinated hydrocarbon insecticides commercially available in 1952 did not give satisfactory control of house flies in the majority of New York dairy barns where they have been previously used. Resistance to lindane, the only material recommended, occurs practically all over the state, although occasionally a barn is found where lindane gives effective control.

With lindane or dieldrin the spot treatment gave more prolonged control than did the overall treatment, but resistance to both materials is common. Resistance to dieldrin was found in one case where no insecticide of any type had ever been used (new barn). Apparently lindane resistant flies had migrated into this barn from a nearby one where the resistance was known to be very high. This would indicate that populations having high lindane resistance may also be resistant to dieldrin. This barn was treated twice with dieldrin, but control was never obtained.

The strip method using dieldrin emulsion gave effective control for 2 seasons in barns where resistance to hydrocarbons was not high. At the present time the strip method is not recommended for barn use in New York State.

Good sanitary conditions are a necessary supplement to any chemical control method if effective control is to be obtained. Any material will give longer periods of control if the barn is relatively free of fly breeding materials.

Summary.—A total of 172 dairy barns were treated during a 2-year period using three application methods. The materials used were lindane wettable powder and emulsion, dieldrin wettable powder and emulsion, DDT wettable powder, DDT wettable powder with K 3936, lindane emulsion with Re 1901, dilan wettable powder, and methoxychlor plus lindane. None of these toxicants tested in the overall and spot methods gave effective control in 1952. The strip methods gave effective control for 2 years if dieldrin had not been previously used, but resistance built up during the third year.

Sanitation is of the utmost importance controlling flies in dairy barns and cannot be stressed too much.

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The Absorption, Distribution and Metabolism of DDT in DDT-resistant Houseflies[1]

A. S. Tahori[2] and W. M. Hoskins

Previous work has shown that certain strains of DDT-resistant houseflies are able to change absorbed DDT into the relatively nontoxic compound DDE, presumably by a process of dehydrohalogenation similar to that which occurs in the presence of strong alkali, though the *in vivo* mechanism of the reaction appears not to have been studied. A second defense mechanism is suggested by the fact that apparently healthy DDT-resistant flies are able to store within their bodies amounts of DDT which would be fatal rapidly to susceptible flies if applied topically to their integument. Almost invariable failure to recover all of the administered dose as DDE plus unchanged DDT indicates that one or more additional degradation reactions may occur to produce compounds that do not respond to the Schechter-Haller test.

The present work was undertaken to gain additional information on the penetration of DDT into the bodies of resistant flies, the accumulation of DDT and of DDE in various tissues at increasing intervals after single and multiple application, and the effect of temperature upon absorption and detoxification of DDT and upon mortality. On the basis of these data an attempt is made to evaluate the relative importance of the three processes which appear to control resistance of the strains studied.

Materials and Methods Used.—The houseflies used were, in order of increasing resistance, the Berkeley, Super Laton and Bellflower strains which were employed in earlier work (Perry & Hoskins, 1950, 1951a and b, Fullmer & Hoskins, 1951, hereafter called I, II and III), plus a moderately resistant Phoenix strain obtained from Arizona. Since most of the strains varied in resistance during the two years period of work, the LD-50's for topical application of acetone solutions of DDT to the ventral thorax are indicated as needed.

The chemicals used were purified p,p′-DDT p,p′-DDE and technical piperonyl cyclonene. The rearing procedures and methods of anesthetizing with carbon dioxide, sexing, application of 1 mm.[3] acetone solutions of toxicant and observation of results were as described in the earlier reports. Only female flies 3 to 5 days old were used except as noted.

Unabsorbed DDT or DDE was removed from the flies by washing with three 15 ml. portions of chloroform which were then combined. The adequacy of removal of unabsorbed toxicant is illustrated by the following data obtained with 82 Phoenix females dead 24 hours after application of 3.50 μg. DDT to the ventral thorax of each.

washing	*total DDT*	*total DDE*	*DDT/fly*[1]	*DDE/fly*[1]
15 ml	36.20	4.26	0.44	0.04
15 ml	7.18	1.13	0.08	0.00 (4)
15 ml	1.29	0.86	0.01	0
15 ml	0.68	0.94	0	0

[1] Corrections for blanks 0.005 μg. apparent DDT, 0.01 μg. apparent DDE per fly were made.

It is clear that recovery of DDT was doubtful in the third portion of solvent. A suggestion regarding the recovery of DDE is given in the discussion. If analysis was to be made for total internal DDT and DDE, the rinsed flies were ground in a mortar in the presence of anhydrous sodium sulfate. The macerated tissues were then extracted by agitating with two 50 ml. portions of chloroform for 20 minutes. Neither longer agitation nor extraction in a Soxhlet apparatus for 6 hours increased the recovery of DDT or DDE, nor was recovery effected by acidification of tissues during extraction.

For determination of DDT and DDE within the various tissues, individual flies were fastened ventral side up to a small piece of filter paper by two minute pins which ran through the thorax and projected into an underlying layer of wax in a petri dish. All further operations were carried out under a binocular microscope. The abdomen was opened with care not to damage the alimentary canal or Mal-

[1] Contribution from the laboratory of insect physiology and toxicology, Department of Entomology and Parasitology, University of California. (Studies on Resistance to Insecticides—IV.)

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pighian tubules. Free liquid was absorbed on pieces of filter paper and, together with any liquid which reached the lower filter paper, was taken as the hemolymph. In numerous cases separation was made into the following parts: "outer tissues" (cuticle and hypoderm plus muscles, legs and wings), "inner tissues" (contents of abdomen such as fat body, ovaries, alimentary canal, Malpighian tubules and hemolymph) and "nerve tissues" (nerve ganglia of head plus thoracic cord). The corresponding body parts from flies of a given experiment were combined and ground with anhydrous sodium sulfate and extracted in the same manner as undissected flies.

Fly tissues contain various chloroform-soluble fatty compounds which interfere with the analysis. These were removed successfully by shaking the chloroform extract with 20 ml concentrated sulfuric acid and then with the same volume of 10 per cent fuming sulfuric acid before proceeding with the nitration procedure.

Analysis for DDT and for DDE was done by the Schechter-Haller procedure and the individual amounts of each were calculated from the optical densities at 530 millimicrons and 600 millimicrons by the two-component color method of Knudson *et al.* (1940). Reference may be made to II for details of the calculations. Since in many analyses very small quantities of DDT and DDE were involved, the slight yellow color which characteristically occurs and increases the optical density at 530 millimicrons to varying extents was very objectionable. This color was traced to a reaction between oxides of nitrogen formed during the nitration procedure and peroxides occurring in the ether used as extracting medium after nitration. Schechter *et al.* (1945) had advocated redistillation of the ether to prevent formation of the yellow color but since ether forms peroxides very quickly upon exposure to air, this procedure was not satisfactory when the highest possible accuracy was needed with minute amounts of DDT or DDE. However, the oxides of nitrogen may be removed and the interfering color almost completely eliminated by placing a suction tube a short distance above the liquid during the last 20 minutes of the one hour nitration period. The spectrophotometer was set to read 100 per cent transmission for the reagents carried through the color development process. Usually cells 1 cm. by 1 cm. by 4.8 cm. were used but when highest sensitivity was needed, micro cells (1 cm. $\times$ 0.3 cm. $\times$ 2.4 cm.) with 0.6 ml. volume of liquid after color development enabled 0.5 micrograms DDT or DDE to be detected. Thus when 50 or more flies were used per test, the analysis was sensitive to 0.01 or less micrograms per fly and in the case of the Berkeley strain, larger numbers enabled amounts in thethird decimal to be determined with less certainty. In all analyses, an equal number of untreated flies or tissues from them were carried through the procedure as a blank. These blanks which ranged from 0.01 μg. apparent DDT and DDE for whole flies down to less than 0.003 μg. for external washings from untreated flies were subtracted in all cases where significant. Results are given in the tables to two decimal places (except for the Berkeley flies) for the sake of consistency in calculating but probably significance is to be attached only to the first decimal place since all errors in application, recovery and analysis affect the final figures.

The DDT brushed off and any DDT, DDE or DDA excreted was determined by analyzing the interior of the container in which the flies were confined after treatment. Either small screen cages or covered beakers could be used but the latter were much more convenient. To determine DDA, the chloroform extract from the cages or beakers was shaken with 2 per cent sodium hydroxide solution (Ferguson & Kearns, 1949) to form the water-soluble salt of DDA. After acidification of the aqueous layer, the free DDA was reextracted with chloroform and analyzed by the Schechter-Haller procedure. It may be stated at once that no appreciable amount of DDA was ever found and consequently this procedure was omitted in the latter part of the work.

RESULTS.—*A. Penetration following application of acetone solutions.*—This subject was not studied systematically but the total amount absorbed can be calculated in nearly all cases as the difference between the DDT applied and the sum of that remaining on the surface of the insects plus that lost to the container. Table 1 gives typical data, in most cases averaged from closely agreeing tests. It may be noted that a) the percentage ab-

sorbed is less as the applied dose increases, b) flies that die during the 24 hours period absorb less than those that survive, c) addition of piperonyl cyclonene lowers absorption of DDT into either survivors or non-survivors, d) the amounts penetrating increased with rising temperature both with and without added piperonyl cyclonene.

A plot of dosage versus per cent absorbed in 24 hours by surviving flies gives a descending curve of decreasing slope which over the range studied may be represented fairly closely by the logarithmic equation, log fraction absorbed $= -k$ dosage. This is the usual relation when the rate of disappearance of the chemical is proportional to the amount present and doubtless applies quantitatively only over a rather limited dosage range. The marked decrease in the fraction absorbed from such a low application as 10 μg. is an indication that the LD 50 values of hundreds or even thousands of μgs. which have been reported for some strains of flies actually refer largely to unabsorbed toxicant. This was stressed by Winteringham (1952) who suggested that a truer comparison of resistant strains might be made on the basis of absorbed DDT required to give 50 per cent mortality.

Loss to the container may be expected to increase with the applied dose and hence contribute to the inefficiency of absorption of larger doses. In the present work, the treated flies were kept in beakers whose contamination was determined. For 25 flies living 24 hours after treatment with DDT the losses were:

dosage in μg./fly	1	1.5	2.0	3.5	4.0	5.0	6.5	10.0	11.7
loss to beaker in μg./fly	0.02	0.03	0.08	0.20	0.24	0.33	0.45	0.72	0.87

Hence the greatest relative loss *i.e.*, 0.87 μg or 0.035 μg. per fly, is small in comparison with the dose and the relatively less efficient absorption of larger doses is not due to loss to the container. When the flies died before the end of the holding period, the loss to the container was slightly less and on the other hand, incorporation of piperonyl cyclonene increased the effect somewhat. All losses to the containers are allowed for, of course, in later calculations of DDT metabolism.

The rate of absorption of DDT by the Phoenix strain at periods up to 24 hours followed the pattern of the other resistant races (cf. II, Table 3). An application of 2 μg. DDT gave the following absorptions. 2 hours (54 per cent), 12 hours (73 per cent), 24 hours (85 per cent), with all flies surviving. Thus the rate of entry is rapid for the first 3 to 4 hours and much slower thereafter. The lower absorption into the Berkeley flies mentioned in II is also illustrated by the data of table 1, *e.g.*, for surviving flies: 0.10 μg., 91 B versus 1.50 μg., 96 P and for those dying within the 24 hour period, 2.00 μg., 58 B versus 2.50 μg., 68 SL.

Table 1 shows that death during the period greatly reduces rate of entry, but a quantitative relation cannot be deduced unless the time of death is known for each fly. However, the 13 per cent absorption in 12 hours by flies previously killed by CO_2 may legitimately be compared with 49 per cent absorption for those which survived application, since the amounts used (10.0 and 10.5 μg.) were almost identical.

DDT penetrates considerably more slowly in the presence of piperonyl cyclonene, probably because the larger molecules of the latter substance enter slowly and meanwhile hold the DDT in solution from which it escapes less readily than when present as the free solid. Several illustrations may be seen in the column for living flies in table 1. In addition, mortality is greatly increased when the synergist is present and penetration at later periods is slowed down accordingly.

The effect of temperature upon absorption of DDT is of special interest in connection with mortality which will be discussed in a later section, but it may be noted that with an application of 5.00 μg. DDT to the Bellflower flies the rates were in the order: 13.5°, 15 per cent; 26°, 58 per cent; 32.5°, 72 per cent. A plot of these data shows a stronger effect of temperature over the lower range.

The last two entries of table 1 show that DDE penetrates readily into Bellflower flies and is held back by piperonyl cyclonene, though the relatively small amount of the latter does not permit quantitative comparison with its effect on entry of DDT.

B. Metabolism of DDT.—A large number of analyses have been made of whole flies following absorption of determined

amounts of DDT after a single application and also after repeated applications at 24-hour intervals. The difference between the amount of DDT which entered and that found at any time permitted calculation of the fraction metabolized. That which was not present as DDE was called X since its nature has not yet been determined. Changes in the amounts of the three substances, DDT, DDE and X, within the flies may be correlated with the development of symptoms of poisoning and subsequent death or recovery. Following application, the absorbed but unchanged DDT increases in amount for several hours during the period when symptoms are developing. Survival seems to depend upon reduction in the amount of DDT whereas those flies which succumb contain even more than during the time of greatest activity. Some typical data are given in table 2, lines 1-2, 3-6, 7-9, 14-17, 18-19, 29-31 and 32-35. The amounts which may be tolerated at 24 hours depend upon the susceptibility of the strain. Average amounts in μgs. found in living and in dead flies at 24 hours are: Berkeley 0.015, 0.028; Super Laton 0.12, 0.38; Bellflower 0.23, 0.43; Phoenix 0.10, 0.14. After 24 hours symptoms of DDT poisoning are not shown usually by houseflies and the unchanged DDT in the body continues to decrease slowly (Table 2, lines 13–16, 23–26 and 29–31). Unless otherwise noted, DDE and X are always expressed as corresponding amounts of DDT in order to facilitate calculations.

Since calculations of the amounts of DDT and DDE in extracts from flies were based upon absorption values at 530 and 600 mμ, corresponding to the peaks for these two substances, the possibility existed that a colored derivative having a peak different from either might be present. Since by hypothesis it would give a lower reading at one or both wavelengths, the calculation would give less than 100 per cent recovery. To check this matter, a group of flies was treated with 5 μg. DDT plus 50 μg piperonyl cyclonene each and after 96 hours analysis was made in the usual manner. A total of 1.80 μg. DDT and 28.60 μg. DDE was calculated from the absorptions at the two wave lengths. In addition thirty-one points on the complete absorption curve were determined.

Three complete absorption curves extending from 320 to 620 mμ were then determined by using 30 μg. DDT, 30 μg. DDE and extract from an equal number of untreated flies. The curves for DDT and DDE were recalculated to 1.80 μg. DDT and 28.60 μg. DDE and composited with that from the untreated flies. The solid curve drawn through the triangles of figure 1 is the result. The 31 points from the first run with poisoned flies are shown by circles. It is obvious that there is complete agreement at the reference wave lengths, 530 and 600 mμ and hence there is no colored derivative having maximum absorption at some nearby wave length. At about 385 mμ some unidentified degradation product has an absorption maximum. All results mentioned have been confirmed with other groups of flies treated with DDT with and without piperonyl cyclonene, but no further study of the unidentified substance has been made.

The amounts of DDE present after application of DDT follow curves of the parabolic type, i.e., rate of increase is greatest in the first few hours and decreases steadily thereafter. In a number of cases a maximum amount of DDE has been found after one to two days and a noticeable loss occurred thereafter. Data on this point were given in Article II and additional data are shown in table 2, lines 10–17, 20–27 and 26–28. This behavior would indicate that DDE is converted into another form which does not respond to the Schechter-Haller test. Additional data on this subject will be given in the sections on repeated dosage and on exposure of larvae and especially in that on direct application of DDE.

On the other hand, X always increases as the flies continue to live. The amounts present at successive periods also follow parabolic curves but the initial increase is considerably steeper than in the case of DDE. Consequently, the ratio X/DDE has high values shortly after application of DDT. These decline to a minimum at about 2 days and then slowly rise due to the simultaneous decrease of DDE and the continuing rise of X (Table 2, lines 7–9, 10 and 12–13, 14–16, 20–23, 26–28, 29–31, and 32–34. The foregoing statements apply to surviving flies. When death occurs, less DDE is formed and usually less X, though not in all cases (Table 2, lines 1–2, 5–6, 10–11, 16–17 and 34–35).

It was mentioned by Perry and Hoskins

(1950) that DDT penetrates into flies killed by heat but is not metabolized, probably because the enzyme required has been inactivated. To test this hypothesis further, Bellflower flies were killed by confining in carbon dioxide for several hours and then were treated with 10.5 μg. DDT plus 30 μg. piperonyl cyclonene. After 12 hours they were analyzed ia the usual manner with the results:

DDT absorbed	*unchanged DDT*	*DDE*	*X*
1.36 μg.	8.81 μg.	0.16 μg.	0.39 μ.

Metabolism was much less than in surviving flies but comparable to that in nonsurvivors; cf. Table 2, Bellflower LD 50=1.8, dying after application of 2.00 μg. DDT.

Temperature has a marked effect upon penetration of DDT (Table 1 and Table 2, lines 20–28). The latter data, which all refers to the application of 5 μg. DDT to Bellflower flies whose LD 50 was 20 μg., show that the unchanged DDT at 24 hours is decreased in later periods and that the X/DDE ratio rises with time at all three temperatures. A point of special interest is the high value of 0.44 μg. DDT in the flies 24 hours after treatment when they appeared to be normal at 32.5°. If cooled to 13.5° or any other low temperature, the flies soon passed into violent agitation and then paralysis. This agrees with the observations of Vinson & Kearns (1952) with roaches.

The Bellflower and Phoenix flies were used for repeated application of DDT at 24 hour intervals to the survivors. This procedure corresponds somewhat to repeated encounters with a treated surface, such as occurs each evening when flies settle for the night. Table 3 gives data on the absorption and metabolism for each application. Those individuals which succumbed in each period were analyzed but, of course, not retreated whereas some of the survivors were analyzed and the rest again treated. These divided themselves in the next period into survivors and nonsurvivors as indicated by the horizontal and inclined lines in the table.

The efficiency of absorption of repeated applications of DDT is as high as that of the first dose as may be noted by inspection or by a plot of the data. Evidently DDT or its metabolites do not alter the permeability of the integument, at least for this substance. Storage of unchanged DDT does not become more important, for the survivors in each interval contain approximately the same amount or usually a little less as time goes on. It may be noted that some of the surviving Phoenix flies contained more DDT than those that succumbed. This behavior was noted often with this strain but not with the others.

DDE increases steadily but less rapidly than X and consequently the ratio X/DDE becomes rather large after three or four days, e.g., 8.01/3.04 or 2.64 for Bellflower flies and 6.70/2.42 or 2.76 for Phoenix flies. If calculated by intervals by the formula $X_i/DDE_i=(X_{t2}-X_{t1})/(DDE_{t2}-DDE_{t1})$, these values are considerably larger. Thus for the Bellflower flies in the 24 to 48 hour interval $(2.38-1.13)\div(1.95-1.04)=1.37$ and similarly 3.30 for 48 to 72 hours, and 7.06 for 72 to 96 hours. The corresponding values for Phoenix flies are 4.01 for 24 to 48 hours and 5.63 for 48 to 72 hours. Mention was made earlier that the X/DDE ratio starts to rise about 2 days after a single application of DDT, by which time all danger from the DDT is past. In the case of repeated applications and hence daily hazard, metabolism to X is considerably increased over that following a single application.

Exposure of larvae to DDT during their feeding period is an extreme type of repeated application. Data have been obtained on three colonies of Bellflower flies reared on a larval diet containing 0.5 per cent DDT which was added as a 50 per cent wettable powder when the diet was prepared prior to introduction of the eggs. With this amount of DDT, larval growth and pupation of the Bellflower strain appear to be practically normal but less than ten per cent of the pupae produced normal appearing flies. Many were unable to emerge completely and some which left the pupal case did not spread their wings. Erratic movements suggestive of DDT poisoning was shown by many for several hours. Similar but less severe symptoms were manifested by flies from larvae kept for the first 3 days only on the DDT-contaminated diet. Analysis of groups of those adults which completed emergence gave the data of table 4. A few tests with larvae and pupae are included. Total absorbed DDT was not determined so values for X cannot be calculated.

The Role of Birds in a Spruce Budworm Outbreak in Maine

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A number of workers have noted that insectivorous birds feed heavily upon larvae and pupae of the spruce budworm, *Choristoneura fumiferanae* (Clem.). Dowden *et al.* (1950) found that birds were one of the important factors in the final reduction of an infestation of this pest in the Adirondacks of New York. Inasmuch as the budworm seemed to be increasing to outbreak proportions in northern Maine in 1949, the Federal Bureau of Entomology and Plant Quarantine and the Fish and Wildlife Service cooperated in a study to measure the effect of birds on the population of the insect.

The field work was done on two moderately infested areas 1.5 miles apart, near Cross Lake, Maine. On one area the birds were not disturbed. On the other an attempt was made to shoot all the birds found during the period they normally feed heavily upon budworm larvae and pupae. The spruce budworm populations on both areas were estimated several times throughout the season in an effort to determine if estimates would indicate the amount the birds had reduced the budworm population in the area where birds were unmolested. This determination was not found to be possible. However, since there did seem to be a difference in percentage reduction due to all natural-control factors in the two areas, the study was repeated in 1950. That year a second area where birds were not disturbed was included as a study area. Estimates of budworm populations in the three areas again failed to provide a reliable quantitative measure of the effect of birds by the methods used, and the study was discontinued. Population estimates, however, were again conducted in two areas in 1951 as a further test of sampling methods, and as part of the regular study program to evaluate the natural-control factors affecting the budworm.

Although the main objective of these studies was not realized, a great deal of information was obtained regarding the number and species of birds present in the areas and the proportion of their diet consisting of spruce budworms. Estimating numbers of birds on the study areas and shooting those found on the experimental area was the function of personnel of the Fish and Wildlife Service.[1] The stomach content of almost all the birds shot on the experimental area was analyzed by Robert T. Mitchell (1952).

It is the purpose of this paper to discuss methods used to estimate spruce budworm populations, and to offer certain conclusions regarding the importance of birds in reducing these populations.

HABITAT.—These studies were conducted in rather typical, second-growth, spruce-balsam areas having a forest composition of about 50-per cent balsam fir, 25-per cent black spruce, 10-per cent white cedar, and 15-per cent miscellaneous hard and soft wood. A large part of each area lay between Cross Lake and Highway 161 in Maine, although the highway cut through the eastern side of the experimental and check area No. 1. Variations in stands are indicated in table 1.

Table 1.—Acreages of various vegetative types on three study areas.

COVER TYPE	EXPERIMENTAL AREA	CHECK AREA No. 1	CHECK AREA No. 2
Spruce-balsam forest:			
Closed	13.5	9½	12
Partially open	17.5	15	9
Brush	7.5	4	7
Highway	1.5	1½	—
Total	40	30	28

Estimates of spruce budworm populations were made on 60 representative balsam trees within the closed spruce-balsam type of each area. The sample trees averaged 37, 39, and 36 feet tall and 5.6, 5.8, and 5.6 inches d.b.h. on the experimental, check area No. 1, and check area No. 2, respectively.

During the winter of 1949–50 a considerable amount of timber was removed from about 1.5 acres of the experimental area east of the highway, but this had little effect on either the species composition or the total population of nesting

[1] Hensley & Cope 1951; Stewart & Aldrich 1951.

birds. In the winter of 1950–51, a power company right-of-way was cut through the area, completely ruining it for further study.

BIRD POPULATIONS.—Systematic sampling indicated that the breeding population ranged from 3.2 to 3.7 pairs per acre with a slight increase in 1950 over 1949. Warblers were the most numerous birds and the bay-breasted warbler was by far the most common species. Among the important species only two showed a marked change in abundance from 1949 to 1950. The olive-backed thrush almost doubled its numbers in 1950, while the magnolia warblers decreased in numbers significantly.

CONTROL OF BIRD POPULATIONS.—Shooting on the experimental area lasted from June 15 to July 8 in 1949 and from June 14 to July 10 in 1950. The number of breeding males was reduced to about 20 per cent of the original population after about a week during both years, and this low level was maintained by continued shooting until the end of the season. A total of 418 adult birds (302 males, 83 females, and 33 sex undetermined) were removed in 1949 and 527 (352 males, 126 females, and 49 sex undetermined) in 1950. A high proportion of the increased number of birds shot in 1950 was made up of sporadically occurring species such as the purple finch, cedar waxwing, white-winged crossbill and gray jay. These four species normally vary greatly in numbers from year to year.

SPRUCE BUDWORMS CONSUMED BY BIRDS REMOVED FROM THE EXPERIMENTAL AREA.—The stomach contents of 826 birds collected on the experimental area were examined. The spruce budworm content of samples collected in 1949 was 21 per cent; in 1950 it was 40 per cent. Among resident birds, warblers, followed by thrushes and sparrows, consumed the greatest number of budworms. Some non-residents also fed heavily upon them. In 1950, 53 non-resident purple finches and 32 non-resident cedar waxwings were collected on the experimental area and budworms were found to comprise about 80 per cent of their food. Since no cedar waxwings were known residents on the experimental area, all that were shot there were considered non residents.

SPRUCE BUDWORM POPULATONS.—The balsam trees used in sampling spruce budworm populations were about 22 feet apart along two or three lines run about 100 feet apart in the central part of each area. In the check areas the same trees were used throughout the study. In the experimental area the lines were changed in 1950, because of the loss of certain trees. Owing to the uniformity of the study areas, however, it is doubted that this appreciably affected the results of the study.

The population of budworms on sample trees was estimated in two ways. By one method, the number of budworms on entire trees was estimated by determining the average number present on twigs 15 inches long, by determining the proportion of the population on these twigs, and by estimating the number of 15-inch twigs per tree.

Twigs 15 inches long or the terminal 15 inches of longer twigs were collected with a pole pruner having a maximum reach of about 35 feet. As they were cut they were dropped to the ground so that they landed on a canvas cloth. When larvae were mining in buds, counts were made on only two or three twigs per tree. How-

Table 2.—Spruce budworms on sample trees along lines in three study areas as determined by collections of 15-inch twigs.

Year	Number of Twigs Collected	Number of Budworms						
		Experimental Area		Check Area No. 1		Check Area No. 2		
		Line 1	Line 2	Line 1	Line 2	Line 1	Line 2	Line 3
		Bud mining Stage						
1949	150	109	136	179	176	—	—	—
1950	120	248	230	359	385	113	96	98
1951	100	—	—	496	418	182	170	144
		Pupal Stage						
1949	600	71	69	107	108	—	—	—
1950	300	77	64	119	96	44	30	28

ever, by the time they had pupated, the population had been reduced to such an extent that five twigs were cut per tree. In 1949 this number was supplemented in the experimental area by cutting five more twigs from adjacent similar trees. Twigs were collected mostly from the lower or midportion of the crown. Observations indicated little difference in intensity of budworm populations at different crown levels.

The spruce budworm infestation was moderate each year, and populations were not heavy enough to cause serious defoliation. Consequently, few larvae wandered about or dropped to the ground. The distribution of large larvae and pupae from the top to the bottom of the crown was rather uniform as determined by the thorough examination of the crowns of two trees in 1950 and seven in 1951. Distribution throughout the area of study was also fairly uniform as determined by comparing collections made on the sample lines (Table 2).

The number of 15-inch twigs per tree was calculated by the formula $y=bx$, where y = the number of 15-inch twigs per tree, b = the crown index (crown length times average crown projection), and $x=1.088484$. The proportion of the population on the 15-inch twigs was determined each year by counting the insects on selected branches cut at different heights on trees in the study areas. In the bud-mining stage 63 per cent of the larvae were found on these twigs in 1949 and 1950, and 67 per cent in 1951; in the pupal stage 65 per cent were on the twigs in 1949 and 1950, and 70 per cent in 1951.

By the second method of estimating budworm populations the number of larvaevorids falling from infested trees was used to determine the number of budworms that had completed larval development. Three species of larvaevorids were used for this purpose—*Phryxe pecosensis* (Tns.), *Aplomya caesar* (Aldr.), and *Lypha setifacies* (West). Each develops as an internal parasite and finally kills the budworm in its full-grown larval or pupal stage. It was possible to use these species for this purpose because when the parasite maggot is full grown it issues and drops to the ground to form a puparium. These maggots were collected on cloth trays 3 feet square, placed under the sample trees. The trays were placed in the same position in the check areas each year. On the experimental area, however, it was necessary to use different trees during the 2 years. These trays provided a satisfactory sample of the entire area under the crowns of the sample trees. This was indicated in 1951 when trays 3 feet wide and as long as the width of the entire crown were used under 15 of the sample trees in the check areas. The number collected on the larger trays was very little different on a unit-area basis from the number collected on the smaller ones.

The trays covered 540 square feet in each area, or 5.4 to 8.1 per cent of the complete crown area of the sampled trees. These figures taken together with percentages of parasitization, as determined by dissections and rearings of larvae, served as the basis for estimating the number of budworms on the sample trees. There was no significant difference in parasitization by larvaevorids at the three study areas.

REDUCTIONS IN BUDWORM POPULATIONS.—The rate of larval development varied considerably. In fact, puparia were recovered for almost 4 weeks, from June 22 to July 17 in 1950.[1] Larvae, therefore, were exposed to parasitization and predation over a considerable period. The pupae were likewise exposed to parasitization and predation over a considerable period, inasmuch as pupal collections were not made until from 20 to 50 per cent of the pupae had produced moths. (Pupae that had produced moths, as evidenced by the fresh pupal skins, were counted as unparasitized pupae.)

Reductions in budworm populations that occurred between the end of larval development and late in the pupal stage were of special interest in this study, because it was during this period that the birds fed most heavily upon budworms. Estimates of population reductions during this period are shown in table 3. Although the cooperative study was not conducted in 1951, data from the two check areas for that year are included.

The number of budworms alive at the end of the larval period was determined by calculating the number of larvae required to produce the puparia recovered on the trays, and reducing this figure by

[1] Birds were removed from the experimental area from June 14 to July 10.

Table 3.—Reductions in spruce budworm populations between full-grown larval and late pupal stages at three study areas (60 sample trees per area).

Year	Number of Budworms Alive at End of Larval Period	Number of Pupae at Time of Collection	Reduction of Budworms		
			Total Number	Per Cent	Average Number per Tree
		Experimental Area			
1949	4,710	4,214	496	11	9
1950	12,945	9,211	3,734	29	63
		Check area No. 1			
1949	26,526	8,191	18,335	69	306
1950	37,756	16,754	21,002	56	350
1951	7,834	3,457	4,377	56	73
		Check area No. 2			
1950	15,115	7,874	7,241	48	121
1951	11,108	3,226	7,882	71	131

the total percentage parasitization of large larvae on the area. The parasites included all larvaevorids and the hymenopteron *Meteorus trachynotus* Vier. The number of pupae at the time of collection was calculated from the average number found per 15-inch twig and the number of 15-inch twigs on the 60 sample trees in each area. (The number of 15-inch twigs was $12{,}799 \pm 1{,}457$, $15{,}125 \pm 1{,}746$ and $15{,}053 \pm 1{,}730$ for the experimental, check area No. 1 and check area No. 2, respectively.)

The important fact brought out in table 3 is the far greater reduction in budworm populations in the check areas where birds were unmolested than in the areas where their numbers were controlled by shooting. It seems logical, therefore, to attribute a considerable portion of the greater reduction in the check areas to feeding by birds. Pupal parasites did not emerge until after the pupal collections were made, and losses through larvae and pupae dropping to the ground were insignificant. The percentage reductions in 1951 at both check plots were similar to reductions in previous years.

Only one other possible factor causing substantial reductions in budworm populations was observed during this period; that was the red squirrel. Twenty-five of these animals (about 80 per cent of the total population) were collected on the experimental area between June 13 and July 10, 1950. Fifteen of them were adult males, nine were adult females, and one was a juvenile. R. T. Mitchell, of the Fish and Wildlife Service, examined the stomach contents and reported that practically all of their diet during this period was insects. Interestingly enough, spruce budworms made up 51 per cent of their total food. The remainder consisted of spruce coneworms, *Dioryctria reniculella* (Grt.), 36 per cent; the spruce bud scale, *Physokermes piceae* (Schr.), 6 per cent; aphids 1 per cent; and undetermined Lepidoptera 4 per cent. An estimate of the number of budworms eaten on the check areas would be largely speculative. However, on the basis of comparative weights it would be possible for a red squirrel to eat 400 to 500 larvae per day. In other words, red squirrels could be important in the reduction of certain budworm populations and they may have caused some of the reduction noted on the check areas in table 3.

Reductions in Budworm Populations on Caged and Uncaged Trees.—It is difficult to check the validity of estimates such as those in table 3. However, some idea of their soundness might be obtained by comparing them with known reductions in populations on several trees in the vicinity of check area No. 1. Eggs were placed on eight small trees, from 10 to 12 feet high, in 1949 and larvae mining in opening buds were counted in the spring of 1950. Two of the trees were caged with muslin cloth, two were caged with quarter inch wire screening, and four were uncaged. Budworms on trees in the cloth-covered cages were protected from birds and parasites, those in wire-screen cages from birds but not from parasites, and those on uncaged trees, of course, were unprotected. Thirteen trees were used in a similar experiment in 1951. Results are summarized in table 4.

It was possible to account for practically all the budworms on trees fully protected by cloth cages. In contrast

Table 4.—Reduction in spruce budworm populations on small caged and uncaged trees in 1950 and 1951.

Year and Type of Cage	Number of Trees	Estimated Number of Budworms at End of Larval Period	Per Cent Mortality of Pupae		Per Cent	
			Dead and Dropped	Parasitized	Moths Emerged	Unaccounted for
1950						
Cloth	2	416	23	0	77	0
Wire	2	218	10	17	37	36
Uncaged	4	208	11	10	24	55
1951						
Cloth	3	437	8	0	89	3
Wire	3	569	15	15	45	25
Uncaged	7	352	1	33	2	64

from 25 to 36 per cent of the budworms on trees protected by wire screening and 55 to 64 per cent on unprotected trees could not be accounted for. They simply disappeared. It seems very important that a much higher proportion of the budworms (20 to 40 per cent) disappeared from unprotected trees where they were exposed to bird predation than from trees protected by wire screening. This fact does not prove that birds caused all the added reduction, but it does lend considerable credence to the theory that they may have been largely responsible.

DISCUSSION.—The importance of bird predators in reducing the number of insects in an area depends upon the number of insects present and other reduction factors. To estimate the effect of birds upon a particular outbreak, it is necessary not only to estimate the number of insects actually consumed, but to relate this to the number actually present.

Certain efforts along these lines have been reported by other workers. Tothill (1923) estimated that in a waning spruce budworm outbreak in New Brunswick in 1918 birds consumed about 13 per cent of the larvae in the area; also that in an outbreak in British Columbia birds accounted for about 39 per cent of the larvae and pupae. These figures are high and indicate great economic importance if applicable over a large area. Kendeigh (1947) estimated that birds destroyed 4.3 per cent of the budworms in a heavy infestation in Ontario in 1945. On the basis of rather general information, he estimated that there were 2,000 budworms per tree, or 376,000 per acre, and that birds consumed 16,000 or the equivalent of 85 budworms per tree. These figures are of considerable interest when compared with those of George & Mitchell (1948), who calculated that the degree of control by birds was from 3.5 to 7.0 per cent in infestations of 500,000 to 1,000,000 budworms per acre. This was equivalent to 35,000 budworms on an acre, slightly more than double Kendeigh's estimate. George & Mitchell based their conclusions on the food requirements of 2.5 pairs of small birds (warblers, etc.) and their nestlings, assuming that during a heavy outbreak budworms were eaten almost exclusively. Mitchell's (1952) studies, based on the food contents of birds shot in the experimental area, would indicate, however, that even in outbreaks heavy enough to supply a complete diet of budworms, only about 40 per cent of the bird's food was made up of this one insect. In this connection it is interesting to note that George & Mitchell (1948) calculated an adult warbler would require 6 grams, or 65 to 100 budworm larvae, a day, Mitchell (1952) found the diet of birds examined in 1950 was 40 per cent budworms, and Kendeigh (1947) believed "a daily consumption of 40 full-grown budworm larvae seems justified."

Estimates made during this study are rather general and they apply to reductions in populations over a period of only about 4 weeks, but it might be of interest to compare them with those referred to above. The limited data from observations made on small caged and uncaged trees (Table 4) indicate that birds removed between one-third and two-thirds of the "percentage unaccounted for." Just what do these figures mean? The heaviest concentration encountered in these studies was located in the check

area No. 1 in 1949 and 1950. Here the total reduction was estimated at about 300 and 350 spruce budworms per tree. Assuming that from one-third to two-thirds of this reduction was attributable to bird predation, between 100 and 200 budworms per tree were destroyed by birds during the periods of observation. The lower figure is somewhat higher than Kendeigh's estimate of 85 per tree. It seems reasonable to believe that his figure was low, however, since he based his calculations on an effective population of only five birds per acre. In Maine the actual number of nesting birds per acre was significantly higher (3.2 to 3.7). In addition, large numbers of budworms were consumed by nonresident birds. For instance, few purple finches and no cedar waxwings nested on the experimental area; yet 64 finches and 32 waxwings were shot there in 1950, and a very high proportion of their diet (79 to 84 per cent) was made up of budworms.

There seems no doubt that birds were important factors in the reduction of the budworm population at Cross Lake. The outbreak was heavy enough to cause 90-per cent defoliation of the current season's growth on many trees in 1950. The removal of possibly 150 large larvae or pupae per tree should have had a marked effect on the number of moths that emerged and laid eggs in the area. It is rather interesting to note that the crown areas of the 60 sampling trees at each study area represented about one quarter acre. A reduction in population of 150 budworms per tree would therefore represent about 36,000 budworms per acre.

It is hardly necessary to point out that in heavy outbreaks the removal of 150 budworms per tree is of little economic significance. Had the Cross Lake infestation been much heavier, complete defoliation would have resulted in spite of the removal of such large numbers of larvae. In light infestations bird predation should be of greater economic importance. Unfortunately, field studies in lighter outbreaks would be difficult to conduct; nevertheless, further research along this line might be of help in determining the factors responsible for the rise and fall of budworm populations.

SUMMARY.—In 1949 and 1950 a cooperative study between the Federal Bureau of Entomology and Plant Quarantine and the Fish and Wildlife Service was undertaken in an attempt to measure the effect of feeding by insectivorous birds on the spruce budworm, *Choristoneura fumiferana* (Clem.), infestation in northern Maine. Field work was carried out on three study areas. On two of them birds were undisturbed. On the other an attempt was made to shoot all the birds found dur,ng the period they normally feed heavily upon budworms. Estimates of budworm populations were made on all three areas before shooting and directly afterwards. While it was impossible to obtain a reliable quantitative measure of the effect of birds by the methods used, indications are that birds consumed between 100 and 300 budworms per tree on one of the check areas in 1949 and 1950. Information obtained regarding the number and species of birds and the food they consumed on the collection area has been published separately.

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The Isolation of Gyptol, the Sex Attractant of the Female Gypsy Moth

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The gypsy moth, *Porthetria dispar* (L.), is a serious pest of fruit, shade, and woodland trees in New England. Large sums of money are spent annually for its control (Burgess 1940) in that area.

Collins & Potts (1932) found that male gypsy moths are attracted by scent to traps baited with a benzene extract prepared from the abdominal tips clipped from virgin female moths. Working with these benzene extractives, Haller *et al.* (1944) observed that the bait was more effective after catalytic hydrogenation, and at the present time this modified bait is used extensively in scouting traps. In addition, they confirmed the observation of W. R. Bloor (Collins & Potts 1932), that the sex attractant is present in the unsaponifiable fraction. Furthermore, they found that the attractant is an alcohol, since it occurred in the phthalic acid ester fraction isolated from the unsaponifiable fraction, but that it was attractive to male moths only after the alcoholic portion had been liberated by hydrolysis of the ester. The digitonin-precipitable part of the alcoholic portion consisted mostly of cholesterol and was found to be unattractive to male moths. The sex attractant, for which the name "gyptol" is suggested, remained in the filtrate. The low yield and low attractiveness to male moths of the recovered gyptol fraction, together with the partial loss of attractiveness observed by Collins and Potts (1932) to have occurred during storage of benzene extracts of moth tips, indicated that uncombined gyptol might be somewhat unstable.

A study of the isolation and properties of gyptol has been continued. Although many of the results described herein are preliminary, nevertheless, in view of the world-wide interest in naturally occurring attractants and repellents, which assume greater importance because of insect resistance to insecticides, it is felt that a description of this work in the nature of a progress report might prove to be of considerable value.

Additional attempts to isolate the attractant have been confined, for the most part, to its derivatives prepared from the unsaponifiable fractions isolated as previously described (Haller *et al.* 1944) from abdominal tips clipped in 1943 and 1945. Various fractions derived from these experiments were tested in the field[1] as attractants to male moths during the flight seasons of 1944 through 1947, at the equivalent concentration of 30 moth tips per 30 ml. of benzene solution per trap. The results of these tests confirmed comparable data obtained previously by Haller *et al.* (1944). The work was considerably handicapped, because the tests were not quantitative and could be made only during the short summer flight of male moths.

Formerly, in the preparation of small quantities of the benzene extractive with commercial benzene, the solvent was removed from the benzene extract of the tips at 50 to 60° C., p = 20 mm., without a detectable amount of the gyptol being volatilized. However, in a much larger quantity of extractive in 1943 a considerable portion of higher boiling material resembling light lubricating oil remained after distillation under these conditions, and it was partly removed at 90° C., p = 1–2 mm. The distillate was found to be somewhat attractive and the necessity for using absolute benzene for extraction was shown. The unsaponifiable fraction was then distilled at 0.2–0.3 mm., but the attractant was found to be spread over several fractions having different characteristics. This method of isolation has not been explored more extensively, because it is felt that gyptol is more heat-labile than could be shown in the absence of a quantitative test and because it was difficult to distill fractionally the small quantities of material that were available.

Attempts to acetylate, benzoylate, and methylate the mixture of alcohols contained in the unsaponifiable fraction have been unsuccessful.

Succinic anhydride proved to be a more suitable esterifying reagent than phthalic anhydride, which was used previously.

[1] The tests were made through the cooperation of Clifford E. Hood, George C. Tierney, Philip Dowden, and David Crosby.

After it had been allowed to react with the unsaponifiable fraction (24 g.) according to the procedure of Wolfe *et al.* (1941), the crude, partly crystalline succinic acid ester fraction (4.88 g.) containing the gyptyl acid succinate was isolated without difficulty, except from material collected in 1944 and 1946, which unaccountably was deactivated.

A portion of the crude acid esters (1.6 g.) was digested with methanol and the 550 mg. of material that crystallized from solution proved to be mostly cholesteryl acid succinate (m.p. 178–179° C., identified by comparison with authentic substance) together with some occluded attractive material. The semisolid active material recovered from the methanol filtrates, after unsuccessful attempts to effect additional crystallization, yielded a dark-yellow fraction and a light-yellow fraction of Skellysolve A percolate when chromatographed on a 2:1 talc-Hyflosupercel mixture. The fact that the most intensely colored fraction seemed to contain practically all the gyptyl acid succinate indicated a correlation between color and attractiveness. (Absorption spectra data in the visible and the ultraviolet[2] regions for this fraction and for more highly purified fractions described below failed to indicate any peak that might be characteristic of gyptol; typical ester spectra were obtained in the infrared.[3]) Further chromatographic separations of the two yellow fractions with both Skellysolve A and carbon tetrachloride showed that correlation between color and attractiveness existed only to indicate the fraction containing activity from the first column, because the yellow fractions obtained from subsequent columns were found by analysis to consist, for the most part, of unattractive compounds of high-molecular weight (approx. 1000 to 1400 by titration) with low-oxygen content. The gyptyl acid succinate fraction (229 mg.) in fact remained on the last columns, and when eluted with warm ethanol it was still contaminated with steroids.

During the delay necessary to obtain biological tests on the ethanol eluate, a more promising procedure was developed. Larger quantities of substances were obtained by combining like fractions from duplicate experiments. The crude acid esters (2 g.) were chromatographed on the talc-Hyflosupercel mixture with Skellysolve A. The yellow effluent was collected as a single fraction and yielded a semicrystalline mixture (680 mg.). Later work with material originally extracted from tips with absolute benzene showed that this chromatographic treatment could be eliminated. Combined fractions of this mixture (865 mg.) gave cholesteryl acid succinate (277 mg.) after it had been dissolved in a minimum of warm benzene and diluted with five volumes of Skellysolve A. The mother liquor residue yielded 32 mg. of unidentified inactive material when digested repeatedly with methanol. The combined methanol solutions, after having been evaporated to one-half and cooled, yielded 57 mg. of amorphous unattractive material. Finally, after the solvent had been completely removed, an attractive viscous oil (463 mg.) was obtained. This oil could not be made to crystallize and an aliquot was sublimed, but the gyptyl acid succinate remained with the steryl acid succinate in the residue. When reacted with monobenzylamine in ethyl acetate solution the oil (89 mg.) gave 12 mg. of the monobenzylamine salt of cholesteryl acid succinate (m.p. 156–158° C.), which immediately crystallized, and the filtrate yielded an oily residue (88 mg.).

One combined lot of this oily residue (518 mg.) was saponified, and the neutral fraction (167 mg.) gave a red color in concentrated sulfuric acid [absorbency index 550 mu (1.4 tips/ml.) = 11.5]. This red color did not appear to be characteristic of gyptol, since the more purified fraction obtained upon chromatographing the benzylamine salts described later gave a lower absorbency index at twice the concentration. The neutral fraction failed to react with semicarbazide hydrochloride in pyridine solution. Cholesterol amounting to 10 per cent of the neutral fraction (85 mg.) was precipitated by digitonin and the oily material (72 mg.) recovered from the filtrate was attractive to moths. This confirmed the observation of Haller *et al.* (1944) that gyptol is not precipitated by digitonin. Incidentally, the oily material showed no effect on the growth of several strains of fungi and bacteria.[4]

[2] Ultraviolet spectrum kindly determined by Harry Bastron, U. S. Bureau of Animal Industry.

[3] Infrared spectra kindly determined by H. L. Cupples of this Bureau and by the Research Laboratory of Merck and Company.

[4] T. D. Fontaine, of the U. S. Bureau of Agricultural and Industrial Chemistry, made the tests on fungi, and E. C. Holst, of the U. S. Bureau of Entomology and Plant Quarantine, the tests on bacteria.

After standing for several months in the refrigerator, this oil (67 mg.) yielded an unidentified colorless crystalline sterol (19 mg., m.p. 109–110° C.), which gave the red color with sulfuric acid but did not attract moths. The separated oily residue (23 mg.) also gave the red color, and it was converted to the colored, partly crystalline *p*-phenylazobenzoate (42.5 mg.) according to Umberger and Curtis (1949), but the gyptyl ester was deactivated during chromatography on florisil. Previously an attempt had been made to use the *p*-phenylazobenzoate as follows: A portion of the attractive neutral fraction obtained by saponification of the crude succinic acid ester fraction was treated with *p*-phenylazobenzoyl chloride by a modification of the method of Mertzweiller *et al.* (1943). The acid chloride was prepared by the method of Ladenburg *et al.* (1938) from acid made according to Baxter *et al.* (1943). The neutral reaction product gave several colored, but inactive, bands on aluminum oxide. The colored benzene-Skellysolve A percolate yielded an attractive residue, but it was deactivated on a second column.

A second portion (357 mg.) of oily benzylamine salts previously described was chromatographed on the talc-Hyflo-supercel mixture with carbon tetrachloride. Two bands developed on the column and consisted of partly crystalline unattractive material. The yellow carbon tetrachloride percolate yielded a viscous oil (299 mg.) that was attractive and gave the red color with sulfuric acid as previously described [absorbency index 550 mu (3 tips/ml.) = 8.1], but it still gave a positive Liebermann-Burchard test for sterols. This oil (296 mg.) was rechromatographed, but instead of being in the yellow percolate, the gyptyl ester fraction (150 mg.) was eluted from the column below the top brown band. Contrary to expectation, the fraction was no longer the benzylamine salt (negative test for nitrogen) and by titration had a neutral equivalent of 591. This material, although still contaminated with steroids, was a highly concentrated fraction of attractive gyptyl acid succinate, from which the alcoholic portion was recovered for chromatographic studies. They will be reported on later.

The partition-chromatographic method of Ramsey and Patterson (1948) for fatty acids was applied first to a portion of the crude succinic acid esters obtained from tips clipped in 1945 and second to a portion of the concentrated fraction of gyptyl acid succinate. In the first case the gyptyl ester was in the second band, which percolated rapidly from the column with Skellysolve A; in the second case it was in the first band (quite broad). In both cases, however, the gyptyl ester was still contaminated with the steryl ester. There was no indication in the second case that further resolution might be obtained by this procedure.

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Spraying the Margins of Fields as a Substitute for Barrier Construction in Chinch Bug Control

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The development of new insecticidal chemicals which are highly toxic to the chinch bug, *Blissus leucopterus* (Say), but safe for use on plants, for the first time makes possible and practical the use of sprays along field margins as a substitute for mechanical or chemical barrier lines. For many years farmers have objected strenuously to the laborious and otherwise trying practice of constructing barriers to prevent chinch bug migration from ripening grain to corn. Now, just as newly developed sprays quickly replaced the use of baits in grasshopper control, it appears quite probable that sprays applied to fence rows and narrow strips in adjacent corn and grain to protect corn from migrating chinch bugs may replace the use of various types of barriers.

The search for new insecticidal chemicals that might be effectively used as sprays to control or prevent chinch bug migrations in Illinois began in 1948. Kearns *et al.* (1949), reported on these preliminary experiments and showed that the gamma isomer of BHC, aldrin, and dieldrin were more toxic to the chinch bug than was dinitro-ortho-cresol; that heptachlor, chlordane, and toxaphene were, in the order named, less toxic than dinitro-ortho-cresol; and that DDT was relatively non-toxic to the chinch bug. Later, in comparable studies, four additional materials formulated as dusts were compared with dieldrin (the outstanding material in the previous tests) by the same technique, that of Decker (1943). In these tests (Table 1) it was found that parathion is apparently slightly more toxic to the chinch bug and faster in its action than dieldrin; that endrin and dieldrin are about equal in toxicity and speed of action; and that dilan and TDE are definitely less toxic to the chinch bug than the other materials tested. In considering both sets of data, dilan appears to be more or less comparable to chlordane, and TDE is about as ineffective as DDT.

In 1949, a light migration of chinch bugs from wheat to corn on a farm in Kankakee County presented a much needed opportunity for preliminary field testing.

At the time these experiments were set up, cinch bugs had already begun to move into the corn, and the first nine or ten rows of corn were moderately infested. Since there were very few bugs beyond the sixth row of corn, plots were set up to include seven rows of corn and 10 feet of the margin of the wheat field, each plot being 0.1 acre in size. The first sprays were applied with a 10-quart, compressed-air type hand sprayer on July 6. On July 7, a second set of plots was established which were the same width, but twice as long, and in this case the spray was applied with a small power sprayer. In both cases the base and underside of the lower leaves of the corn, the soil between the corn rows and between the wheat and corn fields, and the soil and base of the wheat plants in the 10-foot strip of

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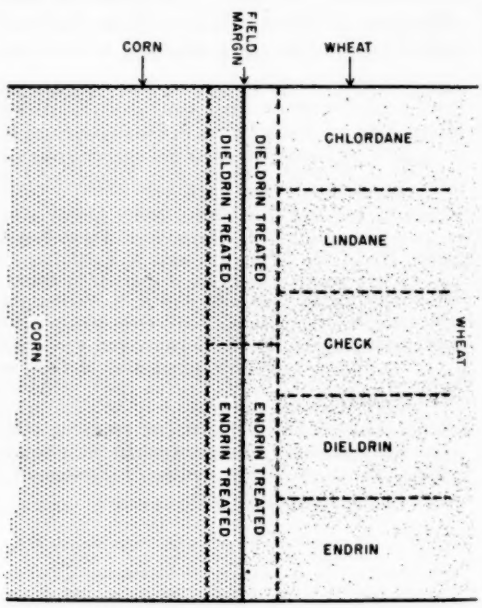

Fig. 1.—Diagram of Field A—1951.

wheat were all sprayed lightly at the rate of approximately 20 to 25 gallons of spray per acre. Materials and amounts used are shown in table 2.

Daily observations were made on all plots for the following 10 days, at which time all migration ceased. Heavy migrations were not observed at any time, even when the wheat was cut on July 8 and 9, but light migrations occurred each day.

Since the data obtained in the two sets of plots are so nearly identical, they are combined and presented in table 2 as the combined results of a duplicated experiment. It is apparent from the data that all of the insecticides tested gave excellent initial kill of the bugs. Only dieldrin, however, gave the long residual kill that is required for success in this type of control operation. There was no evidence that any of the materials was repellent to chinch bugs, since even in the dieldrin plots, bugs were observed moving about after each period of migration. It was considered significant that on the last day of observation most of the insects in the dieldrin plot, including chinch bugs, fungus beetles, chrysopids, ground beetles, lady beetles, and others, were moribund, whereas few if any such insects were observed in the other plots later than the fourth and fifth days following treatment. Since more exact quantitative data were not readily available, the results of this and later experiments are reported in relative numbers of living bugs present.

In 1951 chinch bug migrations on two farms in DeWitt County made possible an extension of field studies. The materials previously tested in 1949 were included in these tests except that endrin, which had recently become available in sufficient quantity for field testing, was substituted for aldrin. Field A, wheat with a thin stand, had a moderately heavy infestation of chinch bugs, largely in the second and third instars, when experiments were set up on June 25, 1951. It bordered corn for a distance of approximately 60 rods. To allow for four treatments and a check, plots were laid out 12 rods long and 8 rods wide in the wheat. Chlordane, lindane, dieldrin, and endrin, at the rates indicated in table 3, were applied to four of the plots, and the fifth was left as a check. All materials were applied with a tractor-drawn power sprayer, using 10 gallons of solution per acre at 75 pounds pressure. Mindful of the short life demonstrated by chlordane and lindane in 1949, the first eight rows of corn and a 2-rod wide strip in adjacent portions of the wheat were sprayed with dieldrin and endrin, 30 rods each, to prevent invasion of the corn field.

Treated areas were examined at convenient intervals, and notations were made on the relative abundance of bugs in the various plots. Two inches of rain fell on these plots on June 27, 2 days after treatment, and about 1 inch fell on July 8. About 75 per cent of the bugs were

Table 1.—Toxicity of dust formulations of some organic compounds applied to adult chinch bugs. Twenty bugs used in each replicate.

Compound	Per Cent Conc.	Replicates	Per Cent Dead			
			1st Day	2nd Day	3rd Day	4th Day
Parathion	1.0	4	92.0	97.8	100.0	
	0.5	4	89.8	100.0		
	0.25	6	85.5	99.2	100.0	
	0.125	10	78.1	99.1	100.0	
	0.1	12	75.2	95.8	99.4	100.0
Dieldrin	1.0	8	58.9	97.9	100.0	
	0.5	8	66.3	92.8	98.2	98.2
	0.25	8	45.5	88.9	97.1	98.9
	0.125	8	16.6	77.9	90.0	92.7
	0.1	8	30.6	82.8	94.4	97.5
Endrin	1.0	9	65.9	93.3	98.0	99.4
	0.5	9	33.9	68.4	94.2	97.7
	0.25	9	15.3	65.9	82.1	89.8
	0.125	9	22.2	59.0	71.9	86.9
	0.1	9	6.4	56.0	71.0	78.9
Dilan	2.0	8	16.0	54.6	71.4	90.5
	1.0	8	15.4	39.3	56.4	72.3
	0.5	8	18.6	37.5	56.7	70.1
	0.25	8	6.0	10.8	21.8	40.0
	0.125	8	5.1	8.9	12.9	18.6
TDE	2.0	3	0.0	0.0	8.0	20.3
	1.0	3	1.7	1.7	1.7	7.0
	0.5	2	0.0	5.0	7.5	15.0
	0.25	2	2.5	5.0	5.0	5.0
	0.125	2	0.0	2.5	7.0	7.0
Pyrax Check	100	13	1.3	5.6	7.2	12.3
Absolute Check		12	2.4	5.1	5.5	5.8

Table 2.—Relative numbers of live bugs in plots at intervals after spraying, July, 1949.

Days After Spraying	Chlordane 1.0 lb./A	Aldrin 0.5 lb./A	Parathion 0.25 lb./A	Lindane 0.3 lb./A	Dieldrin 0.5 lb./A
1	Few	Few	None	None	None
2	Many	Few	Few	Few	None
3	Abundant	Many	Many	Many	None
4		Abundant	Many	Many	None
5			Abundant	Abundant	None
6					None
7					Few[1]
8					Few[1]
9					Few[1]
10					Few

[1] Moribund.

Table 3.—Relative numbers of live bugs in plots at intervals after treatment, 1951.

	Field A				Field B		
Days After Treatment	Chlordane 16 oz.	Lindane 4 oz.	Dieldrin 4 oz.	Endrin 8 oz.	Chlordane 24 oz.	Parathion 8 oz.	Dieldrin 8 oz.
1	Many	Few	Few[1]	Few[1]	Few	None	Few[1]
2					Many	Few	None
4	Abundant	Many	None	None	Abundant	Many	None
7	Like check	Abundant	None	None		Abundant	None
12							Few[1]
20							Many[1]

[1] Moribund.

eliminated by June 27, but the numbers remaining were adequate for comparative purposes.

From the time of treatment until the rain of July 8 dissipated the population and observations were discontinued, no healthy, active bugs were observed on the corn or in the dieldrin- or endrin-sprayed strips immediately adjacent to the corn. Observations on the relative efficiency of the various materials under test in the 8 by 12 rod plots extending on out into the wheat field are presented under the heading "Field A" in table 3. Here again, we find all materials tested except chlordane apparently effectively eliminated bugs present at time of spraying. The chlordane and lindane treatments were obviously short-lived, but the effectiveness of dieldrin and endrin persisted through the seventh day, and when the final observations were made a week later, the population in the check plot was so low that significant comparisons were not possible.

Migrations began in Field B on July 15, at which time the field of thin wheat was carrying a moderately heavy infestation of chinch bugs, with all ages represented, but with fourth and fifth instar bugs predominating. On July 16 the corn was sprayed with dieldrin, 0.5 pound per acre, using a tractor-mounted sprayer delivering 10 gallons of spray per acre, and a similar spray was applied to the first 20 feet of wheat adjacent to the corn. Three plots were laid out 2.5 rods wide and 13 rods long in the wheat beyond the protective treatment. These were sprayed with chlordane, parathion, and dieldrin, as indicated under "Field B" in table 3. Observations made at convenient intervals showed that invasion of the chlordane and parathion plots began by the second day following treatment. The chlordane plots were overrun on the fourth day, and the parathion plots on the seventh day following treatment. The dieldrin plot, however, completely resisted invasion for seven days, and on the 12th day, the margin of the treated and untreated areas was rather clearly defined. As shown in table 3, a few bugs had migrated several feet into the treated area, but all were either moribund or in obvious distress. On the twentieth day, consider-

Table 4.—Relative numbers of living chinch bugs on corn and in treated border strips at intervals after treatment where seven fields were commercially sprayed with dieldrin as indicated to control chinch bug migrations, 1952.

	Applied with Power Sprayer					Aerial Appl.	
Days After Treatment	4 oz./A Huntley	8 oz./A Shull	4 oz./A Kane	8 oz./A Kane	8 oz./A Griffin	8 oz./A Murray I	8 oz./A Murray II
1	None	None	None	None	None	Few	Few
2	None	None	None	None	None	None	None
3	None	None	None	None	None	None	None
4	None	None	None	None	None	None	None
7	None	None	[2]	[2]	None	None	None
10	Few[1]	None			None	Few[1]	Few[1]
14	Few[2]	Few[1]			Few[1]	[2]	[2]
20		Few[2]			Few[2]		

[1] Moribund.
[2] Migration terminated.

Table 5.—Duration of effectiveness (in days) of sprayed barrier strips, 1949–52.

	1949	1951A	1951B	1952
Chlordane	1	0	<1	1
Aldrin	2			
Parathion	2		<4	<4
Lindane	2	<4		
Dieldrin	>10[1]	>7[1]		>14[1]
Endrin		>7[1]	20[1]	>14[1]

[1] Migration terminated.

able numbers of bugs could be found 20 feet, or about half way, across the treated area, but most of them at any distance into the plots were obviously affected, and many were moribund or dead. In no case were live bugs found on the corn, even where they quickly moved across the chlordane- and parathion-treated areas, they were stopped by the dieldrin immediately in front of the corn, and at no time did the corn become reinfested. Rainfall during the period of these observations was recorded as .27 inch, July 17; .69 inch, July 22; .20 inch, July 23; and 1.14 inches, July 31.

In 1952 light to moderate infestations developed in a number of fields in central Illinois and, on seven farms where light to moderate migrations occurred, dieldrin, 0.25 or 0.5 pound per acre, as indicated in table 4, was applied to the first eight rows of corn and for 2 rods into the adjacent wheat or stubble. Five of the fields were sprayed with conventional power equipment, but on two of the fields the applications were made by airplane. Observations made at convenient intervals after treatment are recorded in table 4. In all cases, bugs on the corn and in the grain at the time of treatment were eliminated, and reinfestation did not occur during the period of observation, despite the fact that bugs were present in the grain and attempted migration persisted for from 7 to 28 days, depending upon the field in question.

In some small plot treatments set up in 1952, parathion and endrin performed as they had in 1951.

In summarizing the results of various field tests conducted over a period of 3 years (Table 5) one finds that in the order of their increasing residual effectiveness, the materials would be listed as chlordane, aldrin, parathion, lindane, dieldrin, and endrin, with only the last two showing the degree of persistence required in this type of control operation.

To determine whether or not the use of dieldrin on maturing small grain would under any conditions result in contamination of the grain, four plots (two wheat, two oats) were sprayed at the rate of 8 pounds of dieldrin per acre (16 times the anticipated rate). The grain was cut and threshed within a few (1 to 3) hours after treatment, and samples of treated grain were analyzed in our laboratory by the organic chlorine method deivsed by Stepanov with modifications described by Decker *et al.* (1950). The results presented in table 6 show that under such conditions residues ranged from 3.6 to 9.2 p.p.m.

Samples of wheat and oats similarly taken immediately after treatment at

Table 6.—Dieldrin residues in p.p.m. encountered when grain was sprayed at the rates indicated and samples were collected and threshed within 1–3 hours after treatment.

Rate of Application	Analytical Method	Crop	
		Wheat	Oats
8 lb./A.	Chem. org. chlorine	3.6	9.20
		3.7	8.70
0.5 lb./A.	Bioassay	0.20	0.53
		0.31	0.47

Table 7.—Dieldrin residues on grain samples harvested 3 to 7 days after being sprayed with dieldrin at the rate of 0.5 lb. per acre.

Harvest, Days After Treatment	Dieldrin Residue, p.p.m.	
	Oats	Wheat
3	0.1	0.1
3	0.1	0.1
4		0.1
5	0.1	0.1
7	0.1	

Table 8.—Dieldrin residues on the kernels and lower leaves of corn at harvest time following June and July treatment with dieldrin at the rate of 0.5 lb. per acre.

Farm	Days After Treatment	Dieldrin, p.p.m.	
		Kernels	Leaves
1	97	<0.1	1.8
2	97	<0.1	1.6
3	105	<0.1	0.5
4	103	<0.1	1.4
5	100	<0.1	0.12
6	100	<0.1	0.44

the 0.5 pound per acre rate of application were submitted under code numbers to the Julius Hyman & Company Division of Shell Chemical Corporation for analysis by the bioassay method (Sun & Sun, 1952). As shown in table 6, the residues ranged from 0.2 to 0.53 p.p.m. In both instances the residues on oats were about twice those encountered on wheat. It seems probable that on both crops the contamination was in a large measure due to the contact of the grain with the straw in the threshing process. It should be noted that no time was allowed for the dissipation of residues on the straw before threshing.

In view of the fact that dieldrin residues have been shown to diminish rapidly the first few days after treatment (Decker *et al.* 1950, and Decker 1952), the residues encountered were not considered alarming. However, to determine safety beyond any reasonable doubt, samples of grain were obtained at harvest time from the margins of eight fields (four oats, four wheat) treated at the rate of 0.5 pound per acre 3 to 7 days before harvest. These were analyzed by the bioassay method and in all cases, as is shown in table 7, the residues were reported as less than 0.1 p.p.m.

Samples of ear corn and the lower corn leaves present at the time of treatment in late June and early July were collected at harvest time (October 9). The results of residue analysis obtained by bioassay are shown in table 8. As was expected, there was no evidence of dieldrin on the corn kernels but the leaves showed residues ranging from 0.12 to 1.8 p.p.m. Since the leaves selected were those present at the time of treatment and represented only a small fraction of the total mass of the plants, and since only a very small fraction of the plants in a field are treated at all, such residues represent no real hazard, but to be ultra safe, one might suggest that sprayed corn should not be fed to livestock.

SUMMARY AND CONCLUSIONS.—In this and previously reported studies the relative toxicity of 12 insecticidal chemicals to chinch bugs was determined. Ranked in order of descending toxicity they are: parathion, lindane, dieldrin, endrin, aldrin dinitro-ortho-cresol, heptachlor, dilan, chlordane, toxaphene, DDT, and TDE.

When applied as sprays on infested small grain and corn, parathion, lindane, aldrin, chlordane, dieldrin, and endrin killed most or all of the bugs present at the time of spraying, but only dieldrin and endrin had sufficient residual action to prevent reinvasion for more than two to four days.

None of the materials has been tested under conditions of intensive migration, but on the basis of three years' testing with moderate migrations lasting from 7 to 15 days, it appears that dieldrin applied as a spray at the rate of 0.5 pound per acre on strips 1 to 3 rods wide at the margins of corn and adjacent infested grain fields will effectively prevent chinch bug migrations for at least 10–14 days. Endrin, though less thoroughly tested, appears promising and may be equally effective.

Both performance and harvest residue studies indicate that spraying the adjacent margins of grain and corn fields with dieldrin at the rate of 0.5 pound per acre constitutes a safe, economical, and practical means of preventing chinch bug migration from small grain to corn.

This is a progress report and unqualified recommendations for the use of the indicated control practice await testing under severe infestation and approval of use of the materials for this purpose on corn and small grains.

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Evaluation of Insecticides for Pea Insect Control[1]

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As in pea-growing areas elsewhere in the United States, in New York the most important pea insects are the pea aphid, *Macrosiphum pisi* (Kalt.), and the pea weevil, *Bruchus pisorum* (L.). Heavy infestations of the pea aphid occur only sporadically in western New York. However, this aphid is an economic factor annually because of its role as a vector of pea virus diseases. Although the pea weevil is not always generally abundant, relatively small numbers are of economic importance. It is the principal reason for the application of insecticides to peas in western New York. If weevils can be found by sweeping with an insect net, the field is treated, usually by aircraft dust or spray application. The insecticide is applied when about 50 per cent of the blossoms are showing. If, 4 to 5 days after the first application, weevils can still be found, a second is made. Occasionally a third application is necessary. In the past 1 per cent rotenone dust was used, but this has been largely replaced by parathion sprays or dusts. Since the peas in western New York are grown for canning purposes and the vines are fed to livestock, DDT is not used.

Methods.—During the past two years, a number of insecticides have been evaluated for effectiveness against the pea aphid and the pea weevil. Nearly all of these were applied as spray formulations. Sprays were applied with a tractor-mounted low-volume sprayer, as described by Hervey & Gunkel (1952), operated at 80 pounds pressure. A 21-foot brush-type boom with fan-type nozzles spaced 18 inches apart was used. In the 1951 experiments, the insecticides were applied in 25 gallons of water per acre; in 1952, in 15 gallons of water per acre. The dust formulation was applied with a row-crop power duster.

Plots were arranged in a randomized block design. In 1951, the plots were 42 feet wide by 275 feet long, and in 1952, 20 feet by 100 feet. Treatments were replicated three times in 1951 and four times in 1952. Aphid populations were determined by sweeping with an insect net; weevil infestations by examination of samples of peas. A sample of 500 peas (Table 3) was examined in 1951 and a sample of 2,000 (Table 4) in 1952. Sweepings and samples were taken at random along the length of the plots in the center 6 feet.

Results and Discussion.—*Pea Aphid.*—The results of 2 years' evaluations of insecticides for pea aphid control are summarized in tables 1 and 2. The 1951 tests were conducted on peas; the 1952 tests, on alfalfa. In the 1951 tests, the aphid population was declining from natural causes at the time of the second insecticide application and at the time of taking the last record. This sharp natural decline in the aphid population during the test period did not occur in the 1952 tests.

The phosphate insecticides (parathion, malathon, Metacide, Systox and TEPP) gave excellent control, on the whole. Excellent control was obtained with malathon when used at 1.25 pounds actual toxicant (1 quart) per acre. Results were not as good when it was used at 0.5 pound (0.8 pint) per acre, but it was, nevertheless, an effective aphicide even at this reduced dosage (Table 2). The results obtained with TEPP and DDT in 1952 were somewhat better than those obtained by other workers, Davich & Apple, using comparable methods and dosages.

In these experiments (Tables 1 and 2) methoxychlor, dilan, Q-137 and aldrin appeared to be rather useless for pea aphid control. The order of their effectiveness was such that it is not likely to be adequately enhanced by any practical increase in dosage over the dosages used in these tests.

Two new materials, tetra-n-propyl dithionopyrophosphate (DuPont NPD) and 1-isopropyl-3 methyl-pyrozolyl-(5)-dimethylcarbamate (Geigy 340), were tested (Table 2). Tetra-n-propyl dithionopyrophosphate appeared to be slow-acting, but was equal to the other phosphates in the control obtained after four days. The effectiveness of 1-isopropyl-3 methyl-pyrozolyl-(5)-dimethyl-carbamate

[1] Journal Paper No. 914, New York State Agricultural Experiment Station, Geneva, N. Y., November 24, 1952.

Table 1. Effectiveness of various insecticides against pea aphid. Rushville, N. Y.

Treatments applied June 16 and June 22, 1951

Material and Formulation	Rate per Acre	Per Cent Reduction in Population	
		June 19	June 25
Parathion (25% emul.)	1 pt.	94	96
Malathon (50% emul.)	1 qt.	99	99
Metacide (33.3% emul.)	1 pt.	98	99
TEPP (20% emul.)	1 pt.	95	93
DDT (25% emul.)	1 qt.	91	98
Methoxychlor (25% emul.)	1 qt.	38	58
Dilan (25% emul.)	1 qt.	62	81
Q137 (25% emul.)	1 qt.	28	42
		Ave. no. aphids per 10 sweeps	
Controls		338	53

Table 2.—Effectiveness of several insecticides against pea aphid. Geneva, N. Y.

Treatments applied June 2, 1952

Material and Formulation	Rate per Acre	Per Cent Reduction in Population			
		Days After Treatment			
		1	4	11	18
Parathion (25% emul.)	1 pt.	99	98	79	37
Malathon (50% emul.)	0.8 pt.	92	96	66	30
Systox (42% emul.)	0.5 pt.	99	99	79	66
DDT (25% emul.)	1 qt.	90	95	83	49
DuPont NPD (85% emul.)[1]	0.5 pt.	87	98	75	42
Geigy 340 (25% emul.)[2]	1 pt.	83	91	60	34
Aldrin (23% emul.)	1 pt.	26	40	19	20
Metacide (50% emul.)	0.4 pt.	98	98	71	29
TEPP (20% emul.)	1.25 pt.	89	92	66	33
		Ave. aphids per 10 sweeps			
Controls		1746	1248	1265	3224

[1] Tetra-n-propyl dithionopyrophosphate.
[2] 1-isopropyl-3 methyl pyrozolyl-(5)-dimethylcarbamate.

was less than that of the organic phosphates and DDT.

Pea Weevil.—Examination of the data in tables 3 and 4 will show that for any particular material (except malathon, which was used at a lower dosage in 1952) a lesser degree of control was obtained in the 1951 tests than in the 1952 tests. In 1951, the insecticides were applied twice with 6 days between applications. In 1952, three applications were made at 4- to 5-day intervals. It is probable that the poorer maintenance of coverage in 1951 introduced the discrepancy in the control obtained with the same material in the two tests. A fair evaluation of the weevil control of a series of insecticides with diverse properties cannot be obtained without maintaining continuous coverage, unless it is assumed that the rate of movement into a plot is the same for each plot and constant throughout the period of exposure to oviposition by the weevils. This, of course, is not true. The weevil population is seldom evenly distributed within a field. A number of factors influence the number of weevils entering a field at any particular time (Larson *et al.* 1938). In experiments for weevil control, any such fluctuations of the weevil population, in time and area, would tend to

Table 4.—Effectiveness of various insecticides in reducing pea weevil injury. Geneva, New York.

Treatments applied June 26, June 30, and July 3, 1952

Material and Formulation	Rate per Acre	Infested Peas per 2000 Peas	
		Ave. No.	Control
	pints		
Parathion (25% emul.)	1	0.2	99.5%
Malathon (50% emul.)	0.8	6.2	83.9
DDT (25% emul.)	2	4.0	89.6
Methoxychlor (25% emul.)	2	11.5	70.1
DuPont NPD (85% emul.)[1]	0.5	8.8	77.1
Geigy 340 (25% emul.)[2]	1	40.2	—
Aldrin (23% emul.)	2	2.8	92.7
	pounds		
Rotenone (1% dust)	35–40	4.2	89.1
Untreated	—	38.5	—

[1] Tetra-n-propyl dithionopyrophosphate.
[2] 1-isopropyl-3 methyl pyrozolyl-(5)-dimethylcarbamate.

Table 3.—Effectiveness of several insecticides against pea weevil. Rushville, N. Y.

Treatments applied June 16 and 22, 1951

Material and Formulation	Rate per Acre	Weevils per 10 Sweeps			Infested Peas per 100 Pods	
		12	June 19	25	Ave.	Control
Parathion (25% emul.)	1 pt.	—	0.7	0.3	5.0	68%
Malathon (50% emul)	1 qt.	—	0.3	0.0	2.0	88
Metacide (33.3% emul.)	1 pt.	—	1.0	0.0	2.0	88
Dilan (25% emul.)	1 qt.	—	0.7	0.3	4.0	75
DDT (25% emul.)	1 qt.	—	0.3	0.0	10.0	38
Methoxychlor (25% emul.)	1 qt.	—	4.3	1.7	20.0	—
TEPP (20% emul.)	1 pt.	—	3.7	1.7	29.0	—
Q137 (25% emul.)	1 qt.	—	2.7	0.3	21.7	—
Untreated	—	2.5	0.7	0.7	16.0	

obscure differences in the effectiveness of the insecticides, unless adequate insecticidal coverage were maintained throughout the oviposition period. If residual properties are the primary objective of evaluations, the insecticides should be applied once and samples taken periodically thereafter.

Although "sweeping" with an insect net is an adequate method for obtaining a general estimate of the degree of infestation it is not a satisfactory sampling method for use in insecticide evaluation tests such as those reported herein. A more reliable estimate is the per cent of infested peas, which is also the ultimate criterion of the success or failure of a program for pea weevil control. Table 3 shows little correlation between the infestation as determined by sweeping and that determined by examination of a sample of peas. Maintenance of coverage assumes greater importance in evaluation of insecticides for pea weevil control when records are taken by sampling the mature peas than when records are obtained by "sweeping" for weevils soon after application of the insecticides.

Parathion and Metacide gave better control than did DDT or rotenone, two materials longer used for pea weevil control (Tables 3 and 4). Aldrin was found to be effective against the pea weevil. However, it is likely to present a residue problem comparable to that of DDT where use on peas is concerned. Furthermore, unlike DDT and parathion, it is ineffective against the pea aphid (Table 2) and would have to be supplemented by a different insecticide if aphids were numerous. Such a procedure does not seem practical. These difficulties would not necessarily obviate its use on peas grown for seed. Malathon was effective against the pea weevil at 1 quart per acre (Table 3), but when used at a dosage more nearly comparable to that of parathion it was not as effective as the latter (Table 4). However, at 1 quart per acre, it was more effective than DDT.

The effectiveness of tetra-n-propyl dithionopyrophosphate against the pea weevil should be investigated further. Nearly all the infested peas found in samples taken from plots treated with this material were in one replicate. It is difficult to account for the apparent lack of control in this replicate. In the other replicates, including one where the plot treated with this material was adjacent to a heavily infested check plot, excellent control was obtained. Further tests with dilan should also be conducted.

Q-137, 1-isopropyl-3 methyl-pyrozolyl-(5)-dimethylcarbamate and TEPP were ineffective against the pea weevil. Although methoxychlor has been recommended for pea weevil control (Brindley *et al.* 1946) further contemplation of its use for this purpose does not seem indicated in view of the availability of much superior materials.

SUMMARY.—Control experiments on the pea aphid and the pea weevil are reported. Excellent control of the pea aphid was obtained with parathion, malathon, Metacide and Systox. DDT, TEPP and tetra-n-propyl dithionopyrophosphate were somewhat less effective.

Parathion, malathon, DDT, aldrin and rotenone gave good control of the pea weevil. A comparable degree of control was not obtained with methoxychlor. Data are presented which show the necessity of maintaining adequate coverage when evaluating insecticides for pea weevil control.

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Tissue Distribution, Excretion of $C^{14}O_2$ and Degradation of Radioactive Pyrethrins Administered to the American Cockroach[1]

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Several complex organic esters produced by pyrethrum flowers of the family Compositae, *e.g. Chrysanthemum*[6] *cinerariaefolium* (Treviranus) Boccone, are unique among insecticides in the rapidity with which they paralyze insects. These insecticidal constituents of pyrethrum flowers consist mainly of a mixture of the pyrethrins and cinerins, the pyrethrolone and cinerolone esters of chrysanthemum monocarboxylic acid (Pyrethrin I and Cinerin I) and chrysanthemum dicarboxylic acid monomethyl ester (Pyrethrin II and Cinerin II) (LaForge & Barthel 1944, 1945a, 1945b, 1945c, 1947, La Forge & Soloway 1947a, 1947b, and Soloway & LaForge 1947). The many chemical and toxicological researches which have led to our present knowledge of the mode of action of pyrethrum insecticides have been summarized in publications by Gnadinger (1936, 1945), Hoskins (1940), Metcalf (1948), and Brown (1951).

The characteristically rapid paralytic effects of pyrethrins and cinerins suggest at least an initial action on the central nervous system of insects. The recent biosynthesis of radioactive pyrethrins and cinerins by Pellegrini *et al.* (1952), using $C^{14}O_2$, has made it possible to explore anew the mode of action of these insecticides. By administering these radioactive insecticides in various ways to adult American cockroaches, *Periplaneta americana* (L.), the following studies have been made: (a) tissue distribution of the insecticides within the insects, (b) effect of the insecticides upon respiration and the excretion of $C^{14}O_2$ by the insects, and (c) preliminary degradation studies of the insecticides within the insects.

MATERIALS AND METHODS.—The insects used in these investigations were laboratory-reared, male and female adult American cockroaches, *Periplaneta americana* (L.). In several experiments, the differences in response between the two sexes were determined and compared.

Insecticides.—Both radioactive and non-radioactive pyrethrum insecticides were employed in these studies. The radioactive insecticides used were prepared by Pellegrini *et al.* (1952)[6] and were received as a solution in *n*-hexane. As received and labeled, one milliliter of this solution contained one milligram of pyrethrins. The calculated specific activity of these radioactive insecticides was approximately 0.1 microcuries per milligram. It was soon discovered that the administration to the cockroaches of the *n*-hexane solution of the radioactive insecticides caused an abnormally high mortality. This response by *Periplaneta americana* confirmed that reported by Wigglesworth (1942), who, using the Reduviid bug, *Rhodnius prolixus*, found the onset of toxic symptoms produced by pyrethrins and cinerins in oils could be correlated with the boiling point of the oil, the lighter fractions such as hexane acting most rapidly. Thus, in order to reduce the rapidity of toxic action of the *n*-hexane solution and also to increase the amounts of the radioactive insecticides that could be administered, it was necessary to evaporate the *n*-hexane and redissolve the radioactive insecticides in an equal volume of acetone, a solvent which the cockroaches would tolerate to a much greater extent. As will be noted below, the response of cockroaches to acetone alone was also determined.

The non-radioactive pyrethrum insecticides were received as a two per cent, on a weight to volume basis (e.g. 2 gm. pyrethrins per 100 ml.), solution in deodorized kerosene which was equivalent to a 2.5 per cent solution on a weight-to-

[1] Contribution No. 602, Department of Entomology; Contribution No. 487, Department of Chemistry; and Contribution No. 30, Department of Physics. This research was supported in part by funds provided by the U. S. Atomic Energy Commission under Contract No. AT(11-1)-200. This paper was presented at the 64th annual meeting of the American Association of Economic Entomologists, December 15-18, 1952, Philadelphia, Pennsylvania. The authors acknowledge gratefully the help given by Gael Frank and William E. Robbins in conducting these experiments.

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weight basis.[7] The nonradioactive insecticide was used in developing the paper chromatography method of Winteringham (1952) for the separation and detection of pyrethrin-type insecticides and their derivatives and possible metabolic products. The non-radioactive solution of pyrethrins and cinerins was diluted with *n*-hexane to provide a dilution of the insecticides comparable to the *n*-hexane solution of the radioactive insecticide (i.e. 1 ml. = 1 mg. pyrethrins).

Administration of Radioactive Insecticides.—Various methods were used in the experiments reported in this paper to administer the radioactive pyrethrum insecticides to the cockroaches; these were: (a) interspiracular perfusion, (b) injection into the haemocoele, (c) feeding in acetone and corn oil solution, and (d), contact with a residue on a glass surface. Interspiracular perfusion of the acetone solution of the insecticides was accomplished by anesthetizing the cockroaches with CO_2 (Williams 1946), placing the tip of a full micro-pipette (25 or 50 μl. in volume for the tissue distribution experiments and 5 or 10 μl. in volume for the respiration and $C^{14}O_2$ excretion experiments) on one of the mesothoracic spiracles ("first" thoracic spiracle of Richards & Cutkomp 1945), and allowing the acetone solution of the radioactive insecticides to flow slowly through the spiracle, avoiding any overflow onto the insect's body. The micro-pipette was filled and emptied by attaching it to a manually operated, screw-type micropipettor.[8]

Injecting the acetone solution of the radioactive insecticides into the insect's haemocoele was facilitated by first anesthetizing the insect with CO_2 and then fastening the inactive insect lightly with a rubber band to an inclined surface. The insect and supporting surface were placed on top of a screen-covered Büchner filter funnel through which flowed a stream of CO_2 sufficient to keep the insect continuously anesthetized during the injection. A micro-pipette (5 μl. in volume), fitted at the tip with a short length of a number 27 hypodermic needle, was used for all injections. The micro-pipette plus hypodermic needle tip had a total volume of approximately 7.5 μl. as calibrated with the radioactive insecticides. The insecticidal solution was injected into the haemocoele by inserting the tip of the hypodermic needle through the intersegmental membrane between the fourth and fifth abdominal sternites. The fluid in the micropipette was controlled by a manually operated pipettor as described above.

In order to administer orally known quantities of the radioactive insecticides to the cockroaches, the acetone was evaporated from 50 μl. of the insecticidal solution and the residue redissolved with stirring in 50 μl. of corn oil. A radiometric evaluation of the resulting corn oil solution indicated that a high per cent of the radioactivity in the acetone solution was present in the corn oil solution (see footnote b, Table 2). Cockroaches were anesthetized with CO_2 and gently constrained on their back by rubber bands on a surface like that described above for the injection of the insecticides. The corn oil solution of the radioactive insecticides was allowed to flow slowly from a micro-pipette (10 μl. in volume), the tip of which was placed between the mandibles and well within the mouth opening. By controlling the rate of flow carefully with the manual pipettor described above, the cockroach would "swallow" the corn oil solution and even appear to suck the solution from the micro-pipette. Overflow of the corn oil solution onto the insect's body was avoided.

A fourth method of administering the radioactive insecticides to the cockroaches used in these studies consisted of bringing the insects into contact with a residue of the insecticides deposited on a glass surface. To a lesser extent, perhaps, the action of the vapors of the insecticides were also involved in this method. A volume of the acetone solution of the radioactive insecticides was quantitatively pipetted into a 125 ml. suction filtering flask. A few milliliters of acetone were added to increase the volume and the acetone was evaporated using a gentle stream of air, all the while, the flask was rotated to spread the solution as uniformaly as possible over the bottom and lower two-thirds of its inside surface. Subsequently, cockroaches placed in the flask came into contact with the insecticidal residue. A measure of the insecticidal residue picked

[7] Obtained gratuitously through Dr. Joseph B. Moore, Vice President of McLaughlin Gormley King Company, Minneapolis 14, Minnesota.

[8] Adams suction apparatus, No. A-2473X, Clay-Adams Co., Inc., Chicago 8, Illinois.

up by the roaches was obtained by thoroughly rinsing out the flask and evaluating the radioactivity left after the experiment was completed.

Whenever CO_2 was used to anesthetize the insects, especial care was given to eliminate all the effects of the anesthetic before recording data from any of the experiments described below.

Radiometric Measurements.—The principal equipment used for evaluating the radioactivities derived from the C^{14}-labeled pyrethrins and cinerins consisted of a windowless, flow type counter[9] designed specifically for the measurement of soft radiation, such as that received from C^{14}, and conventional decimal scalers.[10] This equipment was operated in an insulated room in which the temperature was maintained at about 80° F. and the relative humidity at about 50 per cent. All samples were mounted for counting in aluminum planchets designed for use in the windowless counter. Two small circles of C^{14}-labeled polystyrene,[11] mounted in aluminum planchets, with counting rates of approximately 1000 and 3000 counts per minute (cpm), respectively as measured with the equipment described above, were used as reference sources.

Tissue Distribution Experiments.—Five male and five female cockroaches were anesthetized with CO_2, quickly weighed on a torsion balance[12] and treated interspiracularly as described above with 40 and 50 micrograms respectively of the radioactive insecticides to determine the internal distribution of the insecticides or their metabolites. Within five minutes after the insecticides were administered, the insects were semi-paralyzed and lying on their back and they remained in this condition until they were dissected approximately four hours after application of the insecticides.

Dissection of the insects, from the dorsal side of the body, included the following procedures. The mesothoracic tegmina and metathoracic wings were cut off close to their point of attachment to the body. The legs were left on the body to avoid loss of radioactivity through bleeding. A longitudinal incision was made along the dorsum and to one side of the heart and aorta from the anus forward to the head. The body on both sides of the incision was pinned back sufficiently to allow for the location and removal of the following tissues: fore-, mid-, and hind-intestine, brain, thoracic ganglia, thoracic muscle, and abdominal fat. These tissues were transferred to tared microbeakers[13] and then placed in a Petri dish containing a moistened piece of cotton to reduce dehydration before weighing which was done rapidly on a torsion balance as cited above. The insect body remaining after dissection, designated as the "remainder", was transferred to an aluminum-foil boat and also weighed. After weighing, all tissues were held temporarily in the freezing compartment of a refrigerator until they could be extracted as described below.

By adding the sum of the various tissue weights to the weight of the insect body remaining after dissection and dividing by the original body weight it was possible to compute the per cent weight loss during dissection and weighing. The dissections outlined above were practiced first on non-treated specimens to acquire skill and speed; every effort was made to complete the dissections in a minimum period of time and still observe good radioisotope working procedures.

The fresh or freshly frozen tissues (in the latter case, they were allowed to remain at room temperature for a few minutes until thawing had occurred) were quantitatively transferred to a two ounce glass mortar and pestle, mixed with anhydrous, C.P. sodium sulfate, and thoroughly ground. One gram of sodium sulfate was used to dehydrate the fore-, mid-, and hind-intestine samples, 0.5 gram was used with the other tissues, and five grams were used with the remainder. After effecting complete maceration, the mixture was extracted with five or more aliquots of glass-distilled acetone. Each small portion of acetone was decanted after extraction into a 5 ml. volumetric flask. Additional acetone was added to bring the total volume to 5 ml. The volumetric flasks containing the acetone extracts were kept in a refrigerator. Dupli-

[9] Model D-46A, Q-gas counter, manufactured by Nuclear Instrument and Chemical Corp., Chicago 10, Illinois.

[10] The following two scalers were employed: (a) Model 166 scaler, manufactured by Nuclear Instrument and Chemical Corp., Chicago 10, Illinois, and (b) Decimal Scaler Model 2000, manufactured by Berkeley Scientific division of Beckman Instruments, Inc., Richmond, California.

[11] The C^{14}-labeled polystryrene was procured from the Oak Ridge National Laboratory, Oak Ridge, Tennessee.

[12] Roller-Smith torsion balances, manufactured by the Roller-Smith Co., Bethlehem, Pennsylvania, with scales of 0–500 mg. and 0–25 mg., the latter balance was used for weighing the dissected tissues. These balances were made available by Dr. R. H. Painter.

[13] MB-51 Microbeakers, 13 mm. high obtained from R. P. Cargille, New York 16, N. Y.

cate or triplicate 100 μl. aliquots of these tissue extracts were pipetted slowly onto aluminum planchets, allowing time for the acetone to evaporate completely, and then assayed radiometrically with the equipment described above. These same tissue extracts also were used for the paper chromatography separations described below.

Respiration and $C^{14}O_2$ Excretion Experiments.—Measurements of the effects of insecticides upon the rate of respiration have been useful in studying their mode of action. The response of insects to poisoning by pyrethrins and cinerins is characterized successively by excitation, convulsions, paralysis, and death. The initial effects of excitation and convulsions are accompanied by an abrupt increase in respiratory rate (Harvey & Brown 1951). By using C^{14}-labeled pyrethrins and cinerins, it has been possible to study the respiratory response to these insecticides by the American cockroach as well as to determine whether any of the radioactive insecticides were detoxified and degraded to $C^{14}O_2$ which could then be precipitated as $BaC^{14}O_3$ and assayed radiometrically.

A continuous air stream was employed to provide CO_2-free air uniformly to groups of cockroaches. The CO_2 respired by the insects was taken up by the stream of air which was passed through a saturated solution of barium hydroxide where the CO_2 was precipitated as barium carbonate. Essentially, the air passed through an apparatus consisting of three flasks, or other suitable containers, with a capacity of 200 to 300 milliliters, a smaller flask (*e.g.* a 250 ml. suction filtering flask) which served as the respiratory chamber for the cockroaches, and two flasks similar to the first two. These flasks were connected by suitable inlet and outlet tubes made of capillary glass tubing (8 mm. o.d.) running through rubber stoppers. The inlet tube always ran to near the bottom of the flask and the outlet tube projected only a short distance through the rubber stopper. Especial care was given to prevent leaks at any place in the system. Similar systems have been described for $C^{14}O_2$ excretion studies with rats (Gould *et al.* 1949).

The air in the apparatus described above successively passed through the following solutions and chamber, 10 per cent sodium hydroxide, saturated barium hydroxide, saturated sodium chloride, the respiratory chamber containing the cockroaches, and two flasks containing saturated, freshly filtered barium hydroxide. Passage of air through the solutions in the first two flasks in the system removed all CO_2 from the air; the saturated sodium chloride solution in the third flask provided an air supply with a rather constant moisture content. The CO_2 expired by the insects was precipitated in the saturated barium hydroxide solution in the first flask after the respiratory chamber and the second container of saturated barium hydroxide merely served as a check on the completeness of the precipitation of CO_2. All experiments performed with this apparatus were run at room temperatures of from 26° to 30° C. A uniform flow of air through the apparatus during an experiment was maintained by regulating the flow of water siphoning from a large reservoir jug provided with a glass stopcock. The inlet connection to the jug was connected with the respiration apparatus. As the water flowed slowly from the reservoir jug, air was drawn through the apparatus. In these experiments the flow of water from the reservoir jug was approximately 15 ml. per minute.

A number of respiration experiments were performed with this apparatus. In most cases, either five male or five female cockroaches were confined in the respiratory chamber of the apparatus for a period of about nine hours. Extraordinary care was given to the elimination of the CO_2 anesthetic effects described above under *Administration of Radioactive Insecticides* before the treated insects were introduced into the respiratory chamber and the barium carbonate collections begun. The precipitated barium carbonate in the saturated barium hydroxide solution usually was collected at hourly intervals for the first three hours after beginning an experiment and then every two hours by replacing the collection flask with a similar one containing a fresh solution of saturated barium hydroxide. Most respiration experiments were run for a total of nine hours. The precipitated barium carbonate was quantitatively poured into an especially designed filter paper precipitation apparatus[14] and the precipitate washed several times with distilled water and absolute ethyl alcohol.

[14] Model E-8A Precipitation Apparatus, obtained from Tracerlab, Inc., Boston 10, Massachusetts.

The resulting barium carbonate discs were carefully dried under infra-red heat lamps and then stored in a desiccator containing calcium chloride until they were weighed and assayed radiometrically using the windowless counter and scalers described above.

The counting rates of the barium carbonate discs were very low and varied from 6 to 22 counts per minute (cpm) above background or more precisely, from 0.41 to 1.59 counts per minute per milligram per square centimeter (cpm/mg./cm^2). A counting time of 30 minutes per sample was generally used. According to Calvin *et al.* (1949, Appendix II, p. 283–292), the statistical behavior of the counting data obtained is due to the random nature of the disintegration process involved. Since individual nuclei decay independently of each other, the laws of probability can be applied to the observed results. Thus one can select a limit for the probability of error and then calculate the limits of error for a particular number of determinations. By selecting a limit for the probability of 0.5000 ("standard error" of Calvin *et al.* 1949, p. 285) one obtains a value for the probability constant "k" of 0.6745. Using this probability constant value and assuming a counting rate, "R", of 10 cpm above background for a time, "t," of 30 minutes, one obtains a percentage counting error (per cent standard deviation), "E per cent," of approximately 4 per cent using the equation: $E\% = 100k/\sqrt{Rt}$ (Calvin *et al.* 1949, p. 287). The self-absorption of the barium carbonate samples was corrected by the following calculations:

Area of $BaCO_3$ disc..... = 2.8 cm.^{2}
Sample thickness..... = wt. in mg./2.8 cm.^{2}
= ——— mg./cm.^{2}
Counting rate of cample corrected for self-absorption.. = (sample thickness) × (correction factor[a])

[a] Correction factors as listed in Appendix V of Calvin *et al.* (1949, p. 317).

Each experiment performed under this category has been identified with an experiment number as entered in the research notebook; for convenience in presenting this information, these numbers also have been used in this paper. Two preliminary experiments were performed to establish the CO_2 output, as measured by precipitated barium carbonate, of five normal, untreated male (Expt. 34) and female (Expt. 41) cockroaches. Because acetone solutions of the radioactive insecticides were employed in these experiments, a third preliminary experiment (Expt. 38) was conducted, using five male cockroaches, to determine the effect of the solvent on the output of CO_2. In this experiment, each cockroach received 5 microliters (μl.) of acetone interspiracularly. A series of experiments were performed to correlate the method of insecticidal administration with the respiratory response of the cockroaches and their ability to convert the radioactive insecticides to $C^{14}O_2$. The effect of administering acetone solutions of the radioactive insecticides interspiracularly to groups of five male (Expts. 31 and 37) and five female (Expt. 33) cockroaches was determined. Two experiments were conducted to determine the effect of injecting acetone solutions of the radioactive insecticides into the haemocoele of five male (Expt. 39) and five female (Expt. 40) cockroaches. Similarly, the effects of feeding acetone (Expt. 35) and corn oil (Expt. 36) solutions of the radioactive insecticides to two groups of five male cockroaches was studied. Lastly, the effect of bringing 10 male cockroaches into intimate contact with a residue of the radioactive insecticides was studied (Expt. 32); to some extent, the vapors of the insecticides may have been involved also in this experiment.

In all the experiments described above, the following data were either recorded or calculated: (a) the average weight of the cockroaches used, (b) the duration of the experiment (usually 9 hours), (c) the CO_2 output, measured as milligrams of barium carbonate per gram of cockroach body weight per hour, and, where applicable, (d) the rate of insecticidal application, (e) the radioactivity of the insecticides administered, and (f) the per cent of radioactive insecticides administered that could be recovered as $C^{14}O_2$.

Insecticidal Resolution and Degradation Experiments.—The customary methods of analysis for pyrethrins have lacked both the sensitivity and specificity required for detecting and identifying microgram quantities of the insecticides involved in these studies. However, Winteringham (1952) has developed a method of reversed phase paper chromatography for the sepa-

ration and detection of pyrethrin-type insecticides which combines great sensitivity (*e.g.* 1 μgm. of pyrethrins and their hydrolysis products) and comparative ease of manipulation.[15] This method has been used to detect microgram quantities of both radioactive and non-radioactive pyrethrins and cinerins and their corresponding keto-alcohols and chrysanthemum acids and to establish R_f values[16] for the intense blue zones developed after treatment of the paper chromatograms with neutral permanganate and benzidine as outlined in the method.

When working with the radioactive insecticides, the radioactivities of the various color zones on the paper chromatograms also have been evaluated radiometrically. Direct counting of the chromatograms on the Whatman No. 1, one-inch paper strips was accomplished by cutting the paper strips into a series of consecutive, two-centimeter sections, centering the first section over the original point of application of the insecticides. Sections were cut from the entire length of the paper strip and extending one or two centimeters beyond the farthest point of travel of the solvent. These 2 by 2.54 cm. sections of filter paper were placed in an empty planchet and evaluated radiometrically with the windowless, flow type counter and scaler as described above under *Radiometric Measurements*. A comparison of the total radioactivity determined by the above counting proceedure with the total radioactivity determined by counting equal aliquots of the radioactive insecticides originally applied to the paper provided a correction factor for self-absorption losses.

The identification of hydrolysis and degradation products of the pyrethrins and cinerins was aided by carrying out paper chromatography studies of the following non-radioactive compounds, using Winteringham's method: fully racemic

[15] The authors are grateful for the assistance given by F. P. W. Winteringham during a visit to Kansas State College in July of 1952 in these techniques of paper chromatography.

[16] "R_f values" can be defined as the ratio between the distances of travel for the zone in chromatography and the pure solvent. These values are especially useful when direct observation of a zone is being made as in paper chromatography.

Table 1.—A summary of tissue distribution of radioactivity following mesothoracic interspiracular administration of C^{14}-labeled pyrethrins and cinerins to five adult male and five adult female American cockroaches, *Periplaneta americana* (L.). The insects were dissected approximately 4 hours after application of the insecticide.

Sex	C^{14}-Labeled Insecticide Administered[a]	Body Weights[b]		Dissected Tissue Data							
					Weights of Tissues[b]				Radioactivity Measurements		
					Average Weights		Range of Weights		Counts per Minute (cpm) (Corrected) per Milligram of Tissue		% of Total Activity per Tissue Weight[d]
		Average	Range	Tissues	Weights	% of Original Body Wt.[c]	Weights	% of Original Body Wt.[c]	Average	Range	
	(μgm.)	(mg.)	(mg.)		(mg.)	(%)	(mg.)	(%)	(cpm./mg.)	(cpm./mg.)	(%)
Male	40	782	705–830	Digestive System							
				Fore-intestine	15	1.9	12–17	1.5– 2.1	59.5	56.0–63.5	20.2
				Mid-intestine	18	2.3	16– 21	2.0– 2.7	4.3	4.1– 4.7	1.8
				Hind-intestine	18	2.4	15– 20	1.8– 2.8	16.3	15.0–18.0	6.7
				Nervous System							
				Brain	1	0.1	1	0.1	31.4	28.0–36.0	0.7
				Thoracic ganglia	4	0.5	3– 5	0.4– 0.6	14.8	13.6–16.3	1.3
				Thoracic Muscle	5	0.6	3– 6	0.4– 0.9	22.6	20.0–28.3	2.6
				Abdominal Fat	12	1.4	7– 17	1.0– 2.1	3.9	3.5– 4.3	1.0
				Remainder	536	68.3	445–600	63.1–72.3	5.4	5.2– 5.5	65.7
Female	50	1147	900–1330	Digestive System							
				Fore-intestine	65	5.7	26–104	2.6– 7.8	14.4	13.0–15.2	16.6
				Mid-intestine	27	2.4	14– 37	1.1– 3.5	2.7	2.5– 2.9	1.3
				Hind-intestine	66	5.8	38– 96	3.2– 8.5	2.9	2.8– 3.0	3.4
				Nervous System							
				Brain	2	0.2	1– 2	0.1– 0.2	30.0	28.0–31.0	1.0
				Thoracic ganglia	3	0.3	3– 5	0.2– 0.4	12.0	11.0–12.5	0.7
				Thoracic Muscle	5	0.4	3– 10	0.2– 1.1	18.0	17.0–19.0	1.6
				Abdominal Fat	15	1.3	11– 20	0.8– 1.6	2.4	2.3– 2.5	0.6
				Remainder	796	69.3	650–970	63.1–72.9	5.3	5.2– 5.4	74.8

[a] The C^{14}-labeled pyrethrins and cinerins had an average counting rate of 135 cpm./μgm. as determined by the methods described in the text.

[b] All tissue weights are "wet weights" as described in the text.

[c] The totals in these columns do not equal 100% because of weight losses in dissection and weighing. See text, Results, for a statement giving these weight losses.

[d] The data in this column are calculated from the following formula:

$$\text{Per cent of total activity per tissue weight} = \frac{\text{Av. tissue wt.} \times \text{av. cpm./mg. for tissue}}{\Sigma\ (\text{Individual av. tissue wts.} \times \text{av. cpm./mg. for tissue})} = \times 100.$$

cinerin I (*dl-cis*-cineronyl-*dl-trans*-chrysanthemate).[17] pyrethrolone,[18] dihydrocinerolone,[18] allethrolone,[18] and the racemic chrysanthemum monocarboxylic acid (*dl-cis, trans*-chrysanthemum monocarboxylic acid).[18] Although the metabolism of racemic cinerin I and chrysanthemum monocarboxylic acid might be different than that of the natural compounds, it is quite probable that their behavior chromatographically would be similar. Dihydrocinerolone and allethrolone, although not occurring in the plant-derived insecticides, were included for comparison with pyrethrolone and cinerolone.

After establishing the usefulness of these methods of reversed phase paper chromatography with both the radioactive pyrethrins and cinerins and the non-radioactive compounds listed above, experiments were conducted using acetone extracts of various tissues from untreated cockroaches and from cockroaches treated with the radioactive and non-radioactive pyrethrins and cinerins. The acetone extracts were prepared according to the procedures described above under *Tissue Distribution Experiments* and 400 or 500 μl. aliquots of the acetone extracts of the fore-, mid-, and hind-intestine, brain, thoracic ganglia, thoracic muscle, and abdominal fat, and 200 μl. aliquots of the remainder were applied to the paper strips for chromatography. These volumes of the acetone extracts were concentrated to 25 or 50 μl. for chromatographic use. In addition to obtaining information from the location of the color zones on the paper chromatograms of all these tissues, radiometric data were obtained also by counting two centimeter sections from the paper chromatograms of the fore-, mid-, and hind-intestine and the remainder as described above.

Winteringham (1952) has indicated that the keto-alcohol and acid portions of allethrin hydrolysis run together on the reversed phase chromatograms and has suggested a method of separation using washed but non-impregnated paper strips and an ascending solution of petroleum ether saturated with 10 per cent aqueous HCl for 10 minutes followed by a solution of petroleum ether saturated with am-

[17] Obtained from Dr. F. H. Harper, King's College, Strand, London, W. C. 2, England.

[18] Obtained from Dr. R. C. Roark, Division of Insecticide Investigations, Bureau of Entomology & Plant Quarantine, U.S.D.A., Beltsville, Maryland.

Table 2.—A summary of respiration and $C^{14}O_2$ excretion experiments with normal and C^{14}-labeled pyrethrins and cinerin treated American cockroaches, *Periplaneta americana* (L.).

Description of Experiments	Expt. No.	Cockroach Data			Duration of Expt.	Av. CO_2 Output $BaCO_3$ per Hr.	Data from Administration of C^{14}-Labeled Insecticides				
		Sex	N	Average Weight			Rate of Insecticidal Administration	Counting Rate of Insecticides Administered	Radioactivity of Insecticides Administere.[a]	% Radioactive Insecticides Excreted as $C^{14}O_2$	% Radioacti.ity Excreted as $C^{14}O_2$ per μgm. Insecticide Per Gm. Body Wt.
				(gm.)	(hrs.)	(mg./gm. body wt.)	(μgm./gm. body wt.)	(epm (corr.)/gm. body wt.)	(μC. C^{14} /gm. body wt.)×$10\mu^5$	(%)	(% Act. /μgm. /gm')
Normal cockroaches	34	M	5	0.842	9	3.90	—	—	—	—	—
	41	F	5	1.10	9	2.83	—	—	—	—	—
5 μl. acetone/roach interspiracularly	38	M	5	0.828	9	4.29	—	—	—	—	—
5 μl. (Exps. 33 & 37) and 10 μl. (Exp. 31) acetone soln. interspiracularly/roach	31	M	5	0.843	9	14.87	11.86	1423	64.0	9.03	0.76
	37	M	5	0.810	9	14.21	6.17	741	33.4	12.14	1.97
	33	F	5	1.420	9	7.13	4.00	484	22.0	8.40	2.10
7.5 & 11 μl. acetone soln. injected/roach	39	M	5	0.885	9	14.11	8.47	1017	45.8	7.70	0.91
	40	F	5	1.19	11	4.47	10.50	1093	56.8	3.10	0.30
5 μl. acetone soln. fed /roach	35	M	4	0.880	9	7.01	6.25	750	33.8	6.80	1.09
10 μl. corn oil soln. fed /roach	36	M	3	0.763	17	7.06	15.00	1965	88.5[b]	2.65	0.18
Residual deposit	32	M	10	0.830	9	13.71	9.00	1085	47.50	8.00	0.89

[a] The microcuries of C^{14} per gm. body wt.×10^{-5} listed in this column are based upon measurements of the C^{14}-labeled pryeth ins and cinerins, using the procedures and equipment described in the text, and for which an average value of approximately 125 cpm/μgm. of insecticides was obtained (c.f. a counting rate of 135 cpm/μgm, as listed in footnote[a] of Table 1.

[b] Each cockroach was fed 10 μl. of the corn oil solution which had an activity of approximately 150 cpm/μl. indicating that a high per cent of the radioactivity in the acetone solution of C^{14}-labeled pyrethrins and cinerins was incorporated in the corn oil solution.

monium hydroxide for 30 minutes. The strips were dried and sprayed with the permanganate and benzidine reagents in the same manner as for the reversed chase paper chromatography. This technique was applied to 25 μl. aliquots of acetone solutions of the following non-radioactive compounds: pyrethrolone, cinerolone, dihydrocinerolone, allethrolone, and *dl-cis, trans*-chrysanthemum monocarboxylic acid and 600 μl. aliquots (concentrated to a 25 or 50μl. volume by evaporation) of the acetone extracts of the tissues from cockroaches treated with the radioactive insecticides and prepared as described above under *Tissue Distribution Experiments.*

RESULTS.—A summary of the distribution of radioactivity following the mesothoracic interspiracular administration of C^{14}-labeled pyrethrins and cinerins to adult male and female American cockroaches is shown in table 1. It can be seen from these data that the fore-intestine in both sexes contained the greatest amount of radioactivity in the digestive system. The brain, thoracic ganglia, and thoracic muscle also contained a high amount of radioactivity when calculated on the basis of counts per minute (corrected) per milligram of tissue. In both sexes, the fore-intestine contained the highest amount of radioactivity when calculated on the basis of per cent of total activity per tissue weight. The remainder of the insects' body after dissection retained approximately two-thirds to three-fourths of the radioactivity originally administered. The percentage recoveries of the radioactivities administered averaged 82 per cent for the five males and 84 per cent for the five females (range: 80 to 86 per cent). By calculating the sum of the individual tissue weights and the remainder, the average per cent weight loss during dissection was found to be 22.5 per cent for the five male cockroaches (range: 19.5 to 27.5 per cent) and 14.6 per cent for the five female cockroaches (range: 7.1 to 22.1 per cent).

The various respiration of $C^{14}O_2$ excretion experiments are summarized in table 2 and figures 1 through 5. The four methods of administering the C^{14}-labeled pyrethrins and cinerins all resulted in a stimulation of the insects and a greater rate of carbon dioxide excretion, measured as barium carbonate, than occurred with normal cockroaches. The groups of five normal, untreated male and female cockroaches excreted carbon dioxide over a 9 hour period at an average rate equivalent to 3.90 and 2.83 mg. $BaCO_3$/gm. body wt./hr. respectively for the males and females (Expts. 34 and 41 in Table 2). Over the same period of time and under the same experimental conditions, except for an initial interspiracular administration of 5 μl. of acetone per cockroach, five males excreted carbon dioxide at an average rate equivalent to 4.29 mg. $BaCO_3$/gm. body wt./hr. (Expt. 38 in Table 2). This volume of acetone seemed to have little effect on carbon dioxide excretion and thus indicated the suitability of acetone, in this volume, as a solvent for administering the radioactive insecticides in some of the other experiments (*e.g.* Expts. 33 and 37). Furthermore, in these three experiments the carbon dioxide excretion, measured as barium carbonate, occurred at a rather uniform rate as can be seen from the graphs of the data in figure 1 (Expts. 34, 41, and 38), figure 2 (Expts. 34 and 38), and figure 5 (Extp. 34).

Summarizing the data from the experiments involving the four methods of administering the C^{14}-labeled pyrethrins and cinerins is complicated by the different rates of insecticidal administration resulting from the choice of pipetting known small, but reproducible volumes of solutions of the insecticides onto or into cockroaches of different weights. The rate of insecticidal administration varied from 6.17 (in Expt. 37) to 15.00 (in Expt. 36) μgm. of insecticides/gm. of body wt. (Expts. 31, 32, 35, 36, 37, and 39 in table 2) in the case of the males and from 4.00 to 10.50 μgm. of insecticides/gm. of body wt. (Expts. 33 and 40 in table 2) in the females.

The increased average excretion of carbon dioxide, measured as barium carbonate, resulting from these insecticidal treatments ranged from 7.01 (in Expt. 35) to 14.87 (in Expt. 31) mg. $BaCO_3$/gm. body wt./hr. in the males (Expts. 31, 32, 35, 36, 37, and 39 in table 2) and from 4.47 (in Expt. 40) to 7.13 (in Expt. 33) mg. $BaCO_3$/gm. body wt./hr. in the females. A comparison of the cumulative barium carbonate produced in several of the experiments is shown in figure 1. The greater carbon dioxide excretion, as measured by $BaCO_3$ production/gm. body

wt./hr., by groups of five male cockroaches to which the radioactive insecticides were administered interspiracularly (Expt. 37) than by injection into the haemocoele (Expt. 39) is shown in figure 2. The greater carbon dioxide excretion, as measured by $BaCO_3$ production/gm. body wt./hr., by a group of five male cockroaches (Expt. 39) than by a group of five female cockroaches (Expt. 40) is shown in figure 4. The carbon dioxide excretion, as measured by $BaCO_3$ production/gm. body wt./hr., is shown for groups of four and three cockroaches fed the radioactive insecticides in acetone (Expt. 35) and corn oil (Expt. 36) solutions in figure 5. In all these experiments the carbon dioxide excretion by pyrethrin- and cinerin-treated cockroaches was both rapid and pronounced in the first 2 or 3 hours after administration of the insecticides.

The per cent of the radioactive insecticides administered which was excreted as $C^{14}O_2$ varied from 2.65 (in Expt. 36) to 12.14 (in Expt. 37) in the males and from 3.10 (in Expt. 40) to 8.40 (in Expt. 33) in the females. The radioactivity of the $C^{14}O_2$ excreted and counted as $BaC^{14}O_3$ is also shown in figures 3, 4, and 5 for the experiments described above. A question was raised whether the C^{14}-labeled pyrethrins and cinerins possessed sufficient volatility to contaminate the air stream and subsequently the precipitated barium carbonate in these respiration experiments. Accordingly, five normal, untreated, male cockroaches were retained in a clean respiration chamber and a flask containing a residue of 50 μgm. of the radioactive insecticides was added to the system after the flask containing the cockroaches and an experiment run as before for a period of 5 hours. The barium carbonate discs from this experiment showed no radioactivity after being washed with distilled water and absolute ethyl alcohol as described above and it was concluded, therefore, that there was no contamination from this source.

A better basis of comparing the per cent of radioactive insecticides administered by various methods that are excreted as $C^{14}O_2$ may be attained by computing the per cent of radioactivity excreted as $C^{14}O_2$ per μgm. of insecticide per gm. of body weight (per cent Act./μgm./gm.). When this is done (Table 2), it appears that the five male and the five female cockroaches which received interspiracular administrations of acetone solutions of the radioactive insecticides at the rates of 6.17 and 4.00 μgm./gm. body wt. (Expts. 37 and 33) displayed the highest rates of $C^{14}O_2$ excretion and differences due to sex were slight (*e.g.* 1.97 and 2.10 per cent Act./μgm./gm. for the males and females respectively). In a similar experiment (Expt. 31) in which five male cockroaches received a higher rate of insecticidal administration interspiracularly, 11.86 μgm./gm. body wt., a suppression of $C^{14}O_2$ excretion occurred (*e.g.* 0.76 per cent Act./μgm./gm.). The next highest rates of $C^{14}O_2$ excretion were observed when a group of four male cockroaches was fed an acetone solution of the radioactive insecticides at the rate of 6.25 μgm./gm. body wt. (Expt. 35 with a value of 1.09 per cent Act./μgm./gm.), when a group of five male cockroaches was injected with an acetone solution of the radioactive insecticides at the rate of 8.47 μgm./gm. body wt. (Expt. 39 with a value of 0.91 per cent Act./μgm./gm.), and when a group of 10 male cockroaches was exposed to a residue of the radioactive insecticides at the rate of 9.00 λgm./gm. body wt. (Expt. 32 with a value of 0.89 per cent Act./μgm./gm.). Injection of an acetone solution of the radioactive insecticides into the haemocoele of five female cockroaches at the rate of 10.50 μgm./gm. body wt. (Expt. 40 with a value of 0.30 per cent Act./μgm./gm.) and feeding a corn oil solution of the radioactive insecticides to three male cockroaches at the rate of 15.00 μgm./gm. body wt. (Expt. 36 with a value of 0.18 per cent Act./μgm./gm.) resulted in the lowest rates of $C^{14}O_2$ excretion. There was an inverse relationship between the rate of insecticidal administration and the rate of $C^{14}O_2$ excretion when computed as above, the higher application rates in these experiments resulted in the lower $C^{14}O_2$ excretion rates.

An example of the resolution of the C^{14}-labeled pyrethrins and cinerins by Winteringham's (1952) method of reversed phase paper chromatography is shown in figure 6. The greatest concentration of radioactivity (42.5 per cent) was associated with the spot extending from R_f 0.12 to 0.22. A larger spot appeared

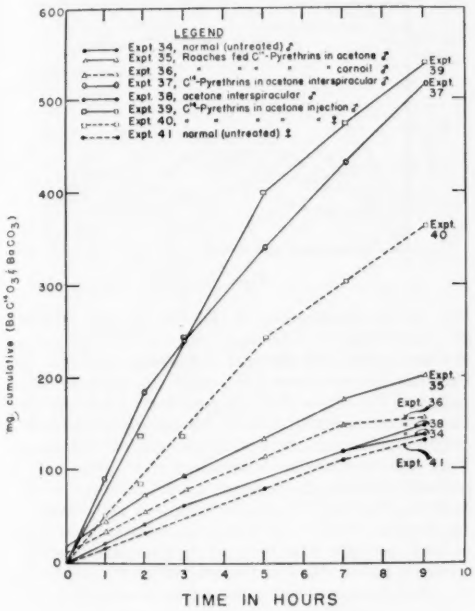
LEGEND
Expt 34, normal (untreated) ♂
Expt 35, Roaches fed C¹⁴-Pyrethrins in acetone ♂
Expt 36, " " " " cornoil ♂
Expt 37, C¹⁴-Pyrethrins in acetone interspiracular ♂
Expt 38, acetone interspiracular ♂
Expt 39, C¹⁴-Pyrethrins in acetone injection ♂
Expt 40, " " " " ♀
Expt 41 normal (untreated) ♀
Expt 39
Expt 37
Expt 40
Expt 35
Expt 36
Expt 38
Expt 34
Expt 41
mg. cumulative (BaC¹⁴O₃ & BaCO₃)
TIME IN HOURS

FIG. 1.—A comparison of the cumulative $BaCO_3$ and $BaC^{14}O_3$ (when radioactive insecticides were administered) produced from the CO_2 and $C^{14}O_2$ excreted by groups of five (except Expts. 35 and 36 where four and three cockroaches were used) male or female American cockroaches, *Periplaneta americana* (L.), treated as described in the legend. The methods of insecticidal administration are described in the text and additional data for these experiments are given in table 2.

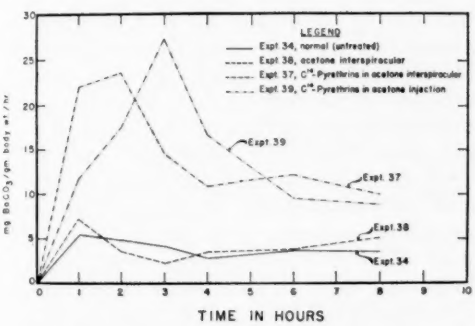

FIG. 2.—A comparison of the $BaCO_3$ and $BaC^{14}O_3$ (when radioactive insecticides were administered) produced from the CO_2 and $C^{14}O_2$ excreted by groups of five male American cockroaches, *Periplaneta americana* (L.), treated as described in the legend. In Expt. 37 the cockroaches received an average of 6.17 μgm. of C^{14}-labeled pyrethrins and cinerins per gram of body weight and in Expt. 39 the cockroaches received an average of 8.47 μgm. of radioactive insecticides per gram of body weight. The methods of insecticidal administration are described in the text and additional data for these experiments are given in table 2.

at R_f 0.49 to 0.67 and contained about 16 per cent of the total radioactivity. These two spots have been designated tentatively as pyrethrin I and II (P I and P II) respectively until the composition of the C^{14}-labeled pyrethrins and cinerins is better known. This conclusion also seems to be in agreement with that expressed by Winteringham (1952). A small, unidentified spot, containing about 4 per cent of the total radioactivity, appeared at R_f 0.1. A large spot developed in the region R_f 0.7 to 1.0. Within this area, two darker-colored blue spots appeared which had a combined radioactivity of about 18 per cent of the total. Good agreement in the location of the spots described above was obtained when a sample containing non-radioactive pyrethrins and cinerins was chromatographed by this method. The following non-radioactive compounds also were chromatographed individually by this reversed phase method and the following R_f values obtained for the center of the spot in each case: fully racemic cinerin I 0.20 and 0.88, pyrethrolone 0.78, cinerolone 0.86, dihydrocinerolone 0.89, alethrolone 0.84, and racemic chrysanthemum monocarboxylic acid 0.89. From these data it would be difficult to separate the keto-alcohol and chrysanthemum acid portions of the pyrethrins and cinerins by this reversed phase method of paper chromatography.

The ascending paper chromatography method, described above under *Insecticidal Resolution and Degradation Experiments*, which utilized separate solutions of petroleum ether saturated with hydrochloric acid and ammonium hydroxide provided an additional means of resolving the esters (pyrethrins and cinerins) and their hydrolysis and degradation products. By chromatographing the C^{14}-labeled pyrethrins and cinerins by this procedure it was found from the location of the blue-colored spots and radiometric counting of the dried paper strip that most of the compounds had run to the end of the solvent travel. This indicated a preponderance of esters (pyrethrins and cinerins) in the radioactive material. Similar studies made with the non-radioactive compounds listed above indicated the value of this procedure for separating and detecting the keto-alcohol and chrysanthemum acid derivatives of

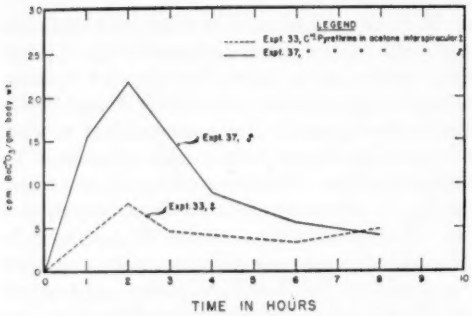

FIG. 3.—A comparison of the radioactivity in the $C^{14}O_2$ excreted by five male and female American cockroaches, *Periplaneta americana* (L.), following mesothoracic interspiracular administration of an acetone solution of C^{14}-labeled pyrethrins and cinerins. The males (Expt. 37) received an average of 6.17 μgm. of the radioactive insecticides per gram of body weight and the females (Expt. 33) received an average of 4.00 μgm. per gram of body weight. The methods of insecticidal administration are described in the text and addutional data for these experiments are given in table 2.

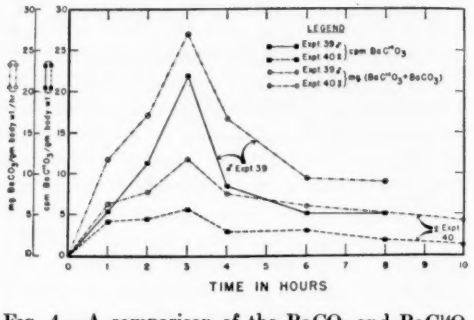

FIG. 4.—A comparison of the $BaCO_3$ and $BaC^{14}O_3$ produced from the CO_2 and $C^{14}O_2$ excreted by groups of five male and female American cockroaches, *Periplaneta americana* (L.), with the corresponding radioactivity of the $BaC^{14}O_3$ produced after injecting an acetone solution of C^{14}-labeled pyrethrins and cinerins into the insects' haemocoele. The males Expt. 39) received an average of 8.47 μgm. of the radioactive insecticides per gram of body weight and the females (Expt. 40) received an average of 10.50 μgm. of the radioactive insecticides per gram of body weight. The method of insecticidal administration is described in the text and additional data for these experiments are given in table 2.

the radioactive pyrethrins and cinerins.

Preliminary studies of the acetone xetracts of tissues from normal and treated cockroaches have resulted in the following tentative conclusions. In applying the reversed phase paper chromatography method to acetone extracts of the fore-, mid-, and hind-intestine, fat, and remainder, an unsaturated, $KMnO_4$-reactive material was always found at or near the point of application ($R_f = 0$). Apparently this spot was similar to that reported by Winteringham (1952) for acetone or neutral ether extracts of macerated house fly tissues. The acetone extracts of all tissues and remainder from normal, untreated cockroaches gave a spot which was centered at about R_f 0.9 to 0.95. This spot interfered with the detection of some of the hydrolysis products of pyrethrins and cinerins as described above. When acetone extracts of tissues from cockroaches treated with the radioactive insecticides were chromatographed by this method the blue bands or spots occurred over a greater range in the region R_f 0.9 and were colored more intensely.

By combining the knowledge gained from both methods of paper chromatography described above and radiometric counting of the paper strips, the following tentative conclusions have been reached regarding the composition of the acetone extracts of the various tissues dissected from cockroaches treated with the radioactive insecticides. The fore-intestine contained a large amount of esters tentatively identified as P I and P II, some keto-alcohols and chrysanthemum acids, and unidentified materials at the point of application and with an R_f value of 0.12. The mid-intestine contained the same compounds as the fore-intestine but in lesser amounts. The hind-intestine contained a slight amount of an ester tentatively identified as P I, a greater amount of an ester tentatively identified as P II, keto-alcohols, a very small amount of the chrysanthemum acids, and unidentified materials at the point of application. The brain contained only hydrolysis products, probably keto-alcohols and chrysanthemum acids, and an unidentified material with an R_f value of 0.12. The thoracic ganglia contained a large amount of an ester tentatively identified as P II, smaller amounts of an ester tentatively identified as P I, and chrysanthemum acids, an unidentified material with an R_f value of 0.12, and apparently no keto-alcohols. The thoracic muscle contained small amounts of an ester tentatively identified as P II, keto-alcohols and chrysanthemum acids, and an unidentified material with an R_f value of 0.12. The remainder contained chiefly the esters tentatively identified as P I and P II, some keto-alcohols and chrysanthe-

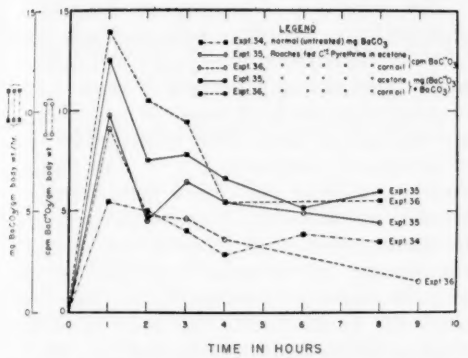

FIG. 5.—A comparison of the $BaCO_3$ and $BaC^{14}O_3$ (when radioactive insecticides were administered) produced from the CO_2 and $C^{14}O_2$ excreted by groups of male American cockroaches, *Periplaneta americana* (L.), with the corresponding radioactivity of the $BaC^{14}O_3$ produced after feeding C^{14}-labeled pyrethrins and cinerins in acetone (Expt. 35) and corn oil (Expt. 36). In Expt. 35 the cockroaches received an average of 6.25 μgm. of the radioactive insecticides per gram of body weight and in Expt. 36 the cockroaches received an average of 15.00 μgm. of radioactive insecticides per gram of body weight. The method of insecticidal administration is described in the text and additional data for these experiments are given in table 2.

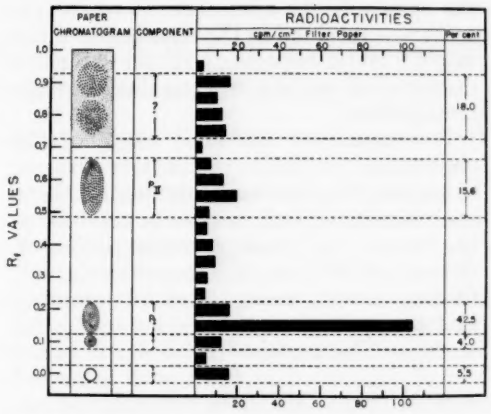

FIG. 6.—A diagrammatic presentation of a reversed phase paper chromatogram (Winteringham 1952) of C^{14}-labeled pyrethrins and cinerins and a histogram of the corresponding radioactivities. A 50 μl.-aliquot of a *n*-hexane solution of the radioactive insecticides (with a counting rate of 6,500 cpm by methods described in the text) was applied at the point $R_f = 0$. The vertical distance on the drawing of the paper chromatogram is greatly shortened in relation to the width; in this particular case the solvent traveled 40 cm. Additional details of this experiment are described in the text.

mum acids, and an unidentified material with an R_f value of 0.12.

DISCUSSION.—It should be pointed out in the tissue distribution experiments that the concentration of radioactivity in the fore-intestine following mesothoracic, interspiracular administration of the radioactive insecticides, as shown in table 1, may be due to the juxtaposition of the fore-intestine in relation to the point of insecticidal application and subsequent perfusion of that portion of the tracheal system. Further tissue distribution studies of the radioactivity from the insecticides, using other methods of administering the insecticides, would help clarify this point.

It was noted in the respiration of $C^{14}O_2$ excretion experiments that all four methods of administering the radioactive insecticides resulted in an abrupt increase in respiratory rate which agreed with the response of the German cockroach, *Blattella germanica* (L.), to pyrethrins as reported by Harvey & Brown (1951).

The susceptibility of insects to pyrethrins and cinerins suggests that their small size denies them a sufficient barrier to delaying the entry of the poison to the vital tissues and also to the fact that their poikilothermic physiology prevents detoxification from operating fast enough to protect them from the poisoning effects of the insecticides. The paper chromatography and radiometric methods which have been employed herein are being used to resolve and identify both the composition of the C^{14}-labeled pyrethrins and cinerins and the degradative metabolites of these compounds in insects. It is anticipated that these techniques can be applied to insecticidally resistant as well as susceptible strains of insects.

SUMMARY.—The tissue distribution and metabolism of C^{14}-labeled pyrethrins and cinerins in the American cockroach, *Periplaneta americana* (L.), have been studied. The internal distribution of the C^{14}-labeled insecticides and their metabolites have been determined in male and female cockroaches approximately four hours following mesothoracic, interspiracular administration of lethal doses of the insecticides. The data from these distribution studies have been summarized in tabular form (Table 1). Of the following dissected tissues: fore-, mid-, and hind-intestine, brain, thoracic ganglia, thoracic muscle, and abdominal fat, the fore-intestine contained the greatest amount of radioactivity. For two-thirds (in males) to three-fourths (in females) of

the radioactivity administered to the insects was retained in the remainder of the insect's body, implying a rather extensive distribution of the insecticides or their metabolites.

A comparison has been made of the conversion of these radioactive insecticides to $C^{14}O_2$ following administration to cockroaches of both sexes via the following routes: (a) interspiracular perfusion, (b) injection into the haemocoele, (c) feeding in acetone and corn oil solutions, and (d) contact with a residue on a glass surface. The highest per cent of radioactivity excreted as $C^{14}O_2$ and measured as $BaC^{14}O_3$ occurred in the case of administration of the radioactive insecticides by interspiracular perfusion. The results of these experiments have been compared both in tabular form (Table 2) and in a series of graphs (Figures 1 through 5).

The detection of microgram quantities of pyrethrins, cinerins, keto-alcohols, and chrysanthemum acids by paper chromatography and the application of these techniques to the study of possible metabolites are presented also. The widespread distribution of radioactivity in the tissues and remainder of the insecticidally-treated cockroaches was confirmed also by radiometric evaluation of paper chromatograms of acetone extracts of the various tissues. Certain tentative conclusions have been reached in these studies which imply the hydrolysis, in the insects, of a large portion of the radioactive pyrethrins and cinerins to corresponding keto-alcohols and chrysanthemum acids. Further work is in progress on this phase of the problem with especial emphasis to the application of these findings to the study of insect resistance to insecticides.

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Variations in Lipid Content of the Boll Weevil and Seasonal Variation in its Resistance to Insecticides[1]

RAYMOND REISER,[2] DANVILLE S. CHADBOURNE,[3] KENNETH A. KUIKEN,[4] C. F. RAINWATER[5] and E. E. IVY[5]

Rainwater & Gaines (1951) have recently demonstrated that the boll weevil, *Anthonomus grandis* Boh, is progressively more difficult to control as the cotton growing season progresses. Chlorinated hydrocarbons were reported to be about 50 per cent as effective in October as in July and calcium arsenate about 67 per cent as effective.

Gaines & Mistric (1951 and 1952) also reported that considerably higher dosages of several chlorinated hydrocarbons were required to give kill of weevils in late September and October to that obtained in August. It was assumed that certain factors other than environmental greatly affected the toxicity of these insecticides. It was shown that the food upon which the weevils developed was partially responsible for the resistance to insecticides late in the season. Because of the small number of generations involved it is unlikely that this is due to the selection of a resistant strain. The possibility remains that some biochemical change occurs in the insect which might be correlated to the month of the year or to a changing nutritional status. The present study is an effort to find what this biochemical change may be.

Because of the more pronounced apparent resistance that develops towards chlorinated hydrocarbon insecticides and the evidence (Long & Fitzhugh 1946; Diephius & Dunn 1949) that such fat soluble materials are concentrated in animal fat, one of the main objectives was to test the concept that the resistance of late season weevils may be due to storage of the poison in an increased amount of body fat.

EXPERIMENTAL.—Weevils were collected from the field during the season from June to November and analyzed for moisture and total lipid. In order to test the relationship between lipid composition and resistance, the results of these analyses were compared with toxicity studies previously and simultaneously made in this laboratory, many of which have been already reported (Rainwater & Gaines 1951). In addition, susceptible and resistant groups (dead and alive at end of test) were analyzed independently and the differences noted.

METHODS.—*Moisture.*—Whole weevils were heated in a vacuum oven at 80° C. for 16 hours. They were then coarsely ground and dried to constant weight in the same oven.

Total Lipid.—The dried samples were extracted by refluxing three times with 20 volumes of 3:1 alcohol-ether mixture, finely ground in a mortar and the extraction repeated twice. The combined filtrates were evaporated to dryness, extracted with petroleum ether, and the extract dried and weighed. The extract was protected with nitrogen at all stages.

Per Cent Net Kill.—The toxicity tests were conducted in field cages according to standard procedures used in evaluating insecticides against the boll weevil. Usually four replicate tests were conducted to complete an individual experiment. Comparable untreated controls were included in each replicate, and the per cent net kill was corrected for the natural mortality occurring in the control cages by means of Abbott's Formula.

RESULTS.—In table 1 are recorded the dry weight and fat content of samples of field collected weevils from June to November 1950 and 1951. These analyses show a steadily increasing fat content as the season progresses,, corresponding rather closely with the reported increase in resistance to toxaphene given in the last column.

In figure 1 the relation of fat content of the weevils and the resistance to toxaphene is graphically illustrated. The significance of the S-shaped curve will be discussed.

In order to determine whether the relation between fat content and resistance

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may be incidental to another, concurrent, factor, the susceptible and resistant (those killed and those remaining alive) weevils from a series of toxicity tests were analyzed (Table 2). These were August field collected weevils.

The figures show conclusively that surviving weevils are pronouncedly fatter than susceptible ones. However, this is true also in the case of the untreated controls. Since these differences are in the same order of magnitude as those between the susceptible and surviving toxaphene and dieldrin treated insects one must conclude that "fatness" is a nonspecific protection which aids survival against any adverse environment. The surviving weevils are also larger and contain more moisture than the susceptible ones.

It was of interest to determine whether the resistance of other cotton insects to toxaphene could be correlated to their body fat. Analyses were therefore made of the larvae of the bollworm, *Heliothis armigera* (Hbn.); saltmarsh caterpillar, *Estigmena acrea* (Drury); fall armyworm, *Laphygma frugiperda* (A. & S.); garden webworm, *Loxostege similalis* (Guen.); yellow-striped army worm, *Prodenia ornithogalli* (Guen.); and cotton leafworm, *Alabama argillacea* (Hbn.). The data are presented in table 3. Last instar larvae were used in all cases to eliminate possible variations due to different degree of maturity. The last instar is the most convenient to work with in the laboratory, though the younger larvae are the most susceptible to insecticidal action. The "toxaphene rating" is in order of their susceptibility, "1" representing the highest degree. No correlation was found.

DISCUSSION.—That chlorinated hydrocarbon insecticides are fat soluble compounds and are readily stored in animal fat depots has been pointed out above. It is a logical assumption, therefore, that the increased resistance of the boll weevil to these poisons, when found to coincide with increased body fat, be attributed to its storage in the fat. Such storage conceivably removes the poison from effective action on whatever metabolic function it normally interrupts.

This concept is strengthened by the S-shape of the curve in figure 1 which shows the relation of the fat content of field collected weevils at different periods and their resistance to a fixed dosage of

Table 1.—Analysis of field-collected weevils compared to per cent killed by toxaphene.

DATE COLLECTED	NO. OF WEEVILS	DRY WT. PER WEEVIL	TOTAL FAT (DRY BASIS)	NET KILL[1]
		mg.	per cent	per cent
6/ 8/51	20	6.6	8.1	98
6/12/51	39	6.6	6.1	
7/26/51	165	5.9	13.9	92
8/11/51	193	6.3	16.2	73
8/20/51	103	6.1	14.9	
9/ 6/50	95	5.9	18.0	47
9/ 9/51	101	8.2	19.1	
10/10/50	130	8.5	22.4	44
10/20/50	176	7.2	24.1	
10/26/50	100	7.8	25.2	
10/26/50	162	7.5	20.6	
11/20/50	100	8.5	22.2	

[1] From Rainwater & Gaines (1951). The values are average figures for the months of June, July, August, September and October.

Table 2.—Total lipid composition of boll weevils susceptible and resistant to various insecticides.

INSECTICIDE		NUMBER OF WEEVILS	AVERAGE WEIGHT PER WEEVIL DRY	TOTAL LIPID (DRY BASIS)	
			mg.	per cent	weighted average per cent
Toxaphene[1]	Susceptible	157	5.4	7.81	—
	Resistant	18	7.2	—	—
Toxaphene[1]	Susceptible	148	6.1	8.56	9.71
	Resistant	23	7.4	17.10	—
Toxaphene[1]	Susceptible	131	5.7	6.46	8.73
	Resistant	22	7.9	22.30	—
Toxaphene[1]	Susceptible	116	5.1	8.65	13.17
	Resistant	43	7.6	25.40	—
Dieldrin[2]	Susceptible	96	6.1	11.70	12.45
	Resistant	13	6.8	18.10	—
Dieldrin[2]	Susceptible	65	5.8	7.70	8.80
	Resistant	17	7.7	13.10	—
Dieldrin[2]	Susceptible	72	6.4	10.50	—
	Resistant	15	7.9	—	—
Dieldrin[2]	Susceptible	66	5.9	6.29	8.23
	Resistant	34	6.8	12.00	—
EPN[3]	Susceptible	125	5.5	7.70	8.47
	Resistant	11	7.5	17.30	—
EPN[3]	Susceptible	130	6.2	13.25	14.87
	Resistant	25	7.4	19.40	—
Methyl parathion[4]	Susceptible	47	5.7	9.50	10.77
	Resistant	31	6.8	12.70	—
Untreated Controls	Susceptible	19	5.3	9.20	11.14
	Resistant	23	7.0	12.80	—
Untreated Controls	Susceptible	14	6.9	9.79	14.34
	Resistant	18	14.4	17.90	—

[1] Applied at the rate of 2 pounds per acre.
[2] Applied at the rate of 0.25 pound per acre.
[3] Applied at the rate of 0.30 pound per acre.
[4] Applied at the rate of 0.15 pound per acre.

Table 3.—The lipids of several species of insects collected from cotton.

Insect	Average Per Cent Fat	Average Iodine Number	Average Per Cent Fatty Acids					Toxaphene Rating[3]
			Linoleic	Linolenic	Arachidonic	Oleic	Saturated	
Boll Weevil[1]	12.8	85.0	8.81	10.15	0.15	37.52	31.37	
Bollworm[2]	20.9	84.5	3.78	23.43	.67	8.20	51.85	2
Salt-Marsh[2] Caterpillar	13.0	105.5	5.17	31.09	.79	12.47	39.60	3
Fall Armyworm[2]	13.1	124.5	15.48	22.10	.00	31.19	17.01	2
Garden Webworm[2]	18.8	111.5	4.26	31.86	.47	12.15	38.86	3
Yellow-striped Armyworm[2]	18.1	97.2	5.99	20.09	.00	30.80	30.92	3
Cotton Leafworm[2]	24.4	132.0	4.90	35.10	.00	26.00	21.77	1

[1] Average of all adult boll weevil analyses made over a 2-year period.
[2] Last instar larvae from cotton.
[3] An estimated relative susceptibility.

toxaphene. The 2 pounds per acre dosage is evidently adequate to kill all weevils with less than 10 per cent fat. The few that escaped this treatment were probably the heavier weevils, the figure of 10 per cent representing only the average. The same is true at the other end of the curve. The susceptible weevils in a mixed population of over 18 per cent fat were probably those containing less than 10 or 12 per cent fat. When this curve is plotted on log-probability paper, it forms a reasonably straight line for this type of data (Fig. 2). The thesis that susceptibility to toxaphene is related to the fat content of the weevil would thus seem to be confirmed. However, by plotting the data of the report of Rainwater & Gaines (1951) against the fat content of weevils in July, August, September and October, an almost identical shaped curve is obtained for those months for calcium arsenate (Fig. 3). One is thus forced to look for a more fundamental condition which can explain both the higher fat content of late season weevils and the greater resistance of late season and fatter weevils to any insecticide.

One possible explanation for fatter weevils as the season progresses may be that there is a larger percentage of older

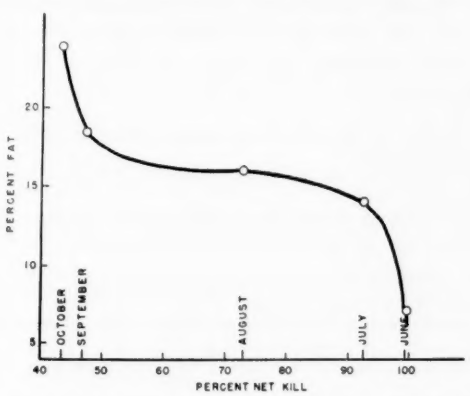

FIG. 1.—The average per cent net kill of 2 pounds of toxaphene per acre versus the average fat content of boll weevils (dry basis) in June, July, August, September, and October.

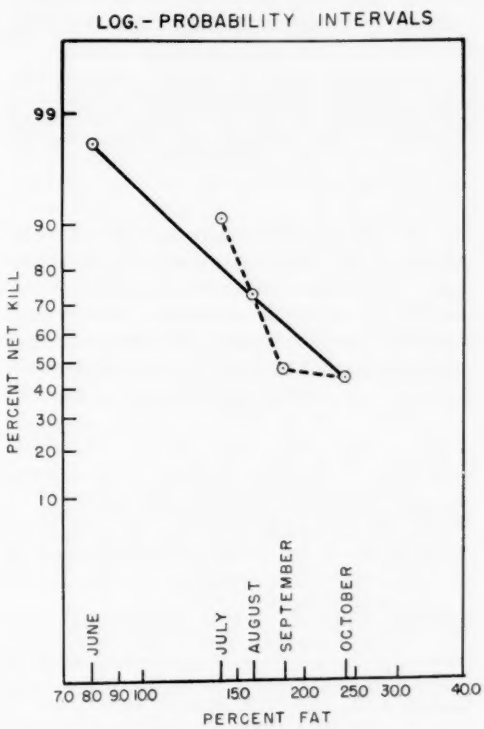

FIG. 2.—The average per cent net kill of 2 pounds of toxaphene per acre versus the average fat content (dry basis) of boll weevils in June, July, August, and September.

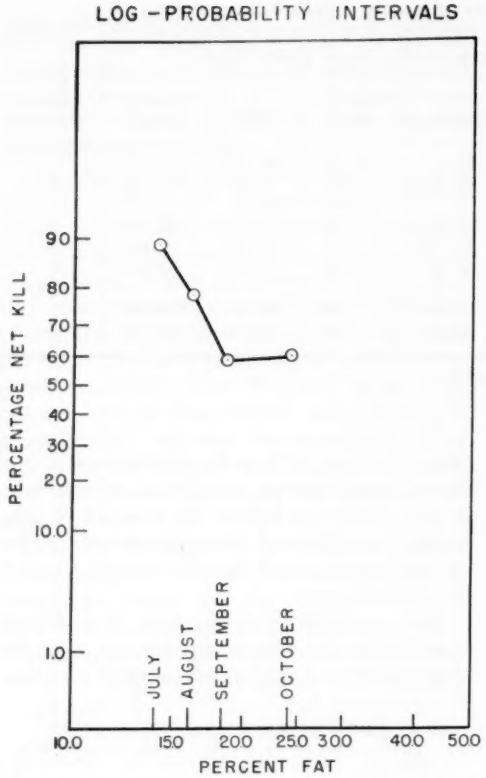

FIG. 3.—The average per cent net kill of undiluted calcium arsenate versus the average fat content (dry basis) of boll weevils in July, August, September, and October.

weevils and a smaller proportion of overwintered weevils. Another explanation may lie in the different nutritional environment of early and late season boll weevils. As the season progresses larger proportions of the insects are boll reared. It is not unlikely that boll reared weevils receive quantitively and qualitatively better nourishment than those reared from squares. Since there is little possibility that adult weevils grow, this condition would more likely explain larger late season weevils than the concept that they are older. Further evidence is obtained from the observation that boll reared weevils are more resistant to insecticides than square reared, Gaines & Mistric (1952).

That resistance to chlorinated hydrocarbon insecticides is not universally related to the percentage of fat in insects is shown by the data presented in table 3. The lipid of a number of insects was compared to their relative susceptibility to toxaphene. No relation was found.

SUMMARY AND CONCLUSIONS.—

1. The total lipid content of the boll weevil increases during the season from a low of about 7 per cent in overwintered to a high of about 22 per cent in October insects.
2. The fat content of the boll weevil is not directly related to its resistance to chlorinated hydrocarbon insecticides as indicated by
   (a) increasing resistance to calcium arsenate during the season and
   (b) the higher fat content of insects resistant to any insecticide tested, or of surviving controls.
3. The relation of high lipid content to high resistance to toxaphene is not a universal quality in other insects.
4. It is suggested that the higher fat content, larger size and increasing resistance of late season weevils may be due to the nutritional advantage of boll reared over square reared insects.

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Effect of Wind and Other Factors on the Toxicity of Certain Insecticides[1]

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Investigations of the effect of climatic factors on the toxicity of certain insecticides have been continued at this station. Results of work conducted during the period 1948 to 1951 have been reported by Gaines & Dean (1949, 1950) and Gaines & Mistric (1951, 1952). High temperatures and humidities, rainfall, dew and sunlight proved to be important factors in reducing the toxicity of certain insecticides.

The experiments herein reported were conducted at College Station, Texas during 1952. Tests were conducted to obtain information regarding the effect of wind and other climatic factors on the toxicity of certain insecticides.

Materials and Methods.—Methyl parathion and heptachlor were included in this study for the first time. The following insecticides were also included in these tests: toxaphene, aldrin, dieldrin, endrin, isodrin, γBHC, malathon, parathion, EPN and TM-1, a Goodrich experimental compound containing a mixture of chlorinated terpene isomers. All of the materials tested were applied as sprays. The sprays were made by diluting miscible oil concentrates with water.

The boll weevil, *Anthonomus grandis* Boh., the cotton leafworm, *Alabama argillacea* (Hbn)., and the salt-marsh caterpillar, *Estigmene acraea* (Drury) were used as test insects. Field collected adult boll weevils were used in all tests. Laboratory reared third instar larvae of the cotton leafworm and salt-marsh caterpillar were used throughout the experiments.

The percentage mortalities were calculated by Abbott's formula. Comparable untreated checks were used in each test. Hygrothermographs were used to record the temperature and relative humidity throughout the investigation. The dosages are shown as pounds of active ingredients per acre. Survival records were made over a period of 5 days following the release of insects onto treated plants.

In the laboratory cage tests the sprays were applied to potted cotton plants. Two hollow cone nozzles were used for dispensing the spray at the rate of approximately 5 gallons per acre. The plants were rotated through the spray as described in previous papers. The sprays were applied at a pressure of 60 pounds per square inch. The percentage mortality shown for each dosage in the laboratory tests represents the average of four replicate cages using from 10 to 15 worms or weevils per cage.

In the tests conducted in the field herein designated as field cage tests, the sprays were applied to individual cotton plants enclosed in a cylinder. A solid cone nozzle dispensing about 7.5 gallons of spray per acre was used. The sprays were applied at a pressure of 60 pounds per square inch. In the field cage tests, the percentage mortality shown for each dosage represents the average of four replicate cages using about 25 weevils per cage.

In some tests the insects were released onto treated plants immediately after treatment, while in other tests the insects were released 24 to 48 hours following the treatment. The insects were released onto plants in all three of the above categories on the same date. This was accomplished by treating the plants involving 24 to 48 hour delay periods 1 and 2 days before the date of the release of insects.

Treated cotton plants exposed to normal climatic conditions outside the laboratory did not receive any rain. A moderate amount of dew was present most mornings during the test periods. The treated plants were exposed to at least medium sunlight. The wind varied considerably during the exposure periods from 2 to 15 miles per hour.

The methods used in making these tests have been described in detail in the papers referred to at the beginning of this article. A method for determining the effect of simulated wind on the toxicity of insecticides was introduced this year. Each lot of treated plants was divided into two groups immediately after the sprays had

[1] Technical Contribution No. 1710, Texas Agricultural Experiment Station in Cooperation with the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. Thanks are due M. E. Merkl and F. M. Fuller for assistance in conducting some of the tests.

dried. One group of potted plants was placed in the greenhouse protected from any wind. The other group of plants also remained in the greenhouse under the same climatic conditions, but these plants were also exposed to the action of the wind. Simulated wind was produced by an ordinary 12-inch oscillating fan. The plants exposed to the wind were arranged in a single row about 3 feet in front of the fan and within the range of oscillation. An anemometer was placed near the plants in an exposed position. An accurate measurement of wind velocity was thus determined over a 24-hour exposure period.

RESULTS.—Experiment 1, (Table 1) was conducted to determine the effect of normal climatic conditions on the toxicity of toxaphene, aldrin, dieldrin and γBHC to the boll weevil. This field cage experiment consisted of 3 series of tests. In the first series the weevils were released immediately after the plants were sprayed, in the second series the weevils were released 24 hours after spraying, and in the third series the weevils were released 48 hours after spraying. Dieldrin retained its toxicity to boll weevils for a longer period of time than did the other insecticides tested. The results of these tests indicate that the normal climatic factors prevailing in the field greatly reduced the toxicity of toxaphene, aldrin and γBHC during a period of 48 hours following treatment.

Experiment 2, shown in Table 2, was conducted to determine the effect of normal climatic conditions on the toxicity of toxaphene, heptachlor, EPN, methyl parathion and endrin to the boll weevil. This field cage experiment consisted of three series of tests which were conducted in the same manner as were those in experiment 1, described above. Toxaphene, EPN and endrin retained their toxicity against boll weevils for a longer period of time than did heptachlor or methyl parathion. These tests indicate that normal climatic factors greatly reduced the toxicity of all the insecticides tested during a period of 48 hours following treatment.

Experiment 3 (Table 3) was conducted in the laboratory to determine the effect of normal climatic conditions on the toxicity of toxaphene, aldrin, dieldrin, and γ BHC when used to control the boll weevil. This experiment also consisted of 3 series of tests. In the first series the weevils were released onto potted cotton plants immediately after treatment. In the second and third series the weevils were released after treated plants had been exposed for 24 to 48 hours to normal climatic conditions outside of the laboratory. The plants in all series were stored in the laboratory for a 5-day period after the weevils were released onto them. Dieldrin retained its toxicity against boll weevils during a longer period of time than did toxaphene, aldrin or γ BHC. However, the toxicity

Table 1.—Results of field cage toxicity tests for boll weevil control using insecticides indicated applied as sprays in experiment 1 at College Station, Texas.

TREATMENT	DOSE[1]	DATE[2]	PER CENT MORTALITY			PER CENT REDUCTION IN TOXICITY IN	
			Immediate Release[3]	24-Hour Delay[4]	48-Hour Delay[5]	24 Hours	48 Hours
Toxaphene	2.0	8-21	63.6	23.1	13.3		
	2.0	10-1	33.4	11.6	20.6		
Average	2.0		48.5	17.4	17.0	64.1	64.9
Aldrin	0.33	8-21	35.0	10.1	4.3		
	.33	10-1	26.3	9.2	14.7		
Average	.33		30.7	9.7	9.5	68.4	69.1
Dieldrin	0.33	8-21	63.2	38.5	46.9		
	.33	10-1	21.3	15.4	15.5		
Average	.33		42.3	27.0	31.2	36.2	26.2
γ BHC	0.33	8-21	46.6	22.6	0		
	.33	10-1	13.6	17.5	7.8		
Average	0.33		30.1	20.1	3.9	33.2	87.0

[1] Pounds active ingredients per acre.
[2] During the test periods, ave. daily temp.: Max. 95° F., Min. 66° F.; relative humidity: Max. 80%, Min. 30%.
[3] Weevils released onto plants immediately after treatment.
[4] Treated plants in field exposed to max. temp. of 101° F. on 8/20 and 92° F. on 9/30. Weevils released onto plants 24 hours after treatment.
[5] Treated plants in field exposed to ave. daily max. temp. of 101° F. from 8/19 to 8/20, and 91° F. from 9/29 to 9/30. Weevils released onto plants 48 hours after treatment.

Table 2.—Results of field cage toxicity tests for boll weevil control using insecticides indicated applied as sprays in experiment 2 at College Station, Texas.

Treatment	Dose[1]	Date[2]	Per Cent Mortality			Per Cent Reduction in Toxicity in	
			Immediate Release[3]	24-Hour Delay[4]	48-Hour Delay[5]	24 Hours	48 Hours
Toxaphene	2.0	8-28	61.7	43.8	21.6		
	2.0	9-4	32.5	17.4	11.6		
Average	2.0		41.1	30.6	16.6	25.5	59.6
Heptachlor	0.33	8-28	63.9	53.6	7.4		
	.33	9-4	17.8	16.9	7.4		
Average	.33		40.9	35.3	7.4	13.7	81.9
EPN	0.33	8-28	84.2	75.5	31.9		
	.33	9-4	77.8	36.9	48.3		
Average	.33		81.0	56.2	40.1	30.6	50.5
Methyl	0.33	8-28	97.5	48.3	19.2		
Parathion	.33	9-4	66.1	37.5	19.0		
Average	.33		81.8	42.9	19.1	47.6	76.7
Endrin	0.33	8-28	96.3	63.1	57.3		
	.33	9-4	73.0	65.8	29.0		
Average	.33		84.7	64.5	43.2	23.8	49.0

[1] Pounds active ingredients per acre.
[2] During the test periods, ave. daily temp.: Max. 95° F., Min. 64° F.; relative humidity: Max. 74%, Min. 28%.
[3] Weevils released onto plants immediately after treatment.
[4] Treated plants in field exposed to max. temp. of 95° F. on 8/27 and 93°F. on 9/3. Weevils released onto plants 24 hours after treatment.
[5] Treated plants in field exposed to ave. daily max. temp. of 97° F. from 8/26 to 8/27 and 93° F. from 9/2 to 9/3. Weevils released onto plants 48 hours after treatment.

of all the materials was greatly reduced during the 48-hour exposure of treated plants to normal climatic conditions.

Experiment 4, (Table 4) was conducted in the laboratory to determine the residual toxicity of toxaphene, heptachlor, EPN, methyl parathion and endrin when protected from normal climatic conditions. Three series of tests were conducted in the same manner as described above in experiment 3, except that the treated plants were retained in the laboratory during a 24 and 48-hour period following treatment. Thus the treated plants were protected from outside climatic conditions. Heptachlor was the only insecticide used that showed an appreciable loss in toxicity when protected from normal climatic conditions.

Experiment 5, (Table 5) was conducted in the laboratory to determine the effect of 0.5 inch of simulated rain on the toxicity of toxaphene, endrin, heptachlor, EPN and methyl parathion to the boll weevil. This experiment consisted of two series of tests. One series consisted of treated plants which received no rain. In the other series 0.5 inch of simulated rain was applied to the treated plants immediately after the sprays were allowed to dry. Heptachlor was the only insecticide in this experiment that showed a loss in

Table 3.—Results of laboratory cage toxicity tests for boll weevil control using insecticides indicated applied as sprays in experiment 3 at College Station, Texas.

Treatment	Dose[1]	Date[2]	Per Cent Mortality			Per Cent Reduction in Toxicity in	
			Immediate Release[3]	24-Hour Delay[4]	48-Hour Delay[5]	24 Hours	48 Hours
Toxaphene	2.0	8-22	88.8	60.0	4.0	32.4	95.5
Aldrin	0.33	8-22	85.6	49.9	0	41.7	100
Dieldrin	0.33	8-22	96.0	83.3	30.3	13.2	68.4
γ BHC	0.33	8-22	40.1	11.0	4.2	72.6	89.5

[1] Pounds active ingredients per acre.
[2] During the test period, ave. daily temp.: Max. 99° F., Min. 72° F.; relative humidity: Max. 81%, Min. 30%.
[3] Weevils released onto plants immediately after treatment.
[4] Treated plants stored outside of the laboratory under normal conditions at max. temp. of 94° F. on 8/21. Weevils released onto plants 24 hours after treatment.
[5] Treated plants stored outside the laboratory under normal conditions at ave. daily max. temp. of 98° F. from 8/20 to 8/21. Weevils released onto plants 48 hours after treatment.

Table 4.—Results of laboratory cage toxicity tests for boll weevil control using insecticides indicated applied as sprays in experiment 4 at College Station, Texas.

Treatment	Dose[1]	Date[2]	Per Cent Mortality			Per Cent Reduction in Toxicity in	
			Immediate Release[3]	24-Hour Delay[4]	48-Hour Delay[5]	24 Hours	48 Hours
Toxaphene	2.0	8-29	97.5	85.4	89.5		
	2.0	9-5	61.5	67.6	79.3		
Average	2.0		79.5	76.5	84.4	3.8	0
Heptachlor	0.33	8-29	94.4	36.7	29.9		
	.33	9-5	72.7	60.5	59.9		
Average	.33		83.6	48.6	44.9	41.9	46.3
EPN	0.33	8-29	100	100.0	100		
	.33	9-5	96.3	100	95.6		
Average	.33		98.2	100	97.8	0	.4
Methyl	0.33	8-29	100	100.0	100.0		
Parathion	.33	9-5	100	100	100		
Average	.33		100	100	100	0	0
Endrin	0.33	8-29	100	100.0	100.0		
	.33	9-5	97.2	100	95.6		
Average	.33		98.6	100	97.8	0	.8

[1] Pounds active ingredients per acre.
[2] During the text periods, ave. daily temp.: Max. 92° F., Min. 79° F.; relative humidity: Max. 76%, Min. 38%.
[3] Weevils released onto plants immediately after treatment.
[4] Treated plants stored inside of the laboratory protected from normal conditions at max. temp. of 94° F. on 8/28 and 94° F. on 9/4. Weevils released onto plants 24 hours after treatment.
[5] Treated plants stored inside of the laboratory protected from normal conditions at ave. daily max. temp. of 94° F. from 8/27 to 8/28 and 94° F. from 9/3 to 9/4. Weevils released onto plants 48 hours after treatment.

Table 5.—Results of laboratory cage toxicity tests for boll weevil control using insecticides indicated applied as sprays in experiment 5 at College Station, Texas.

Treatment	Dose[1]	Date[2]	Per Cent Mortality		Per Cent Reduction in Toxicity Due to Rain
			No Rain[3]	Simulated Rain.[4]	
Toxaphene	2.0	8–13	100	81.7	
	1.5	8–25	95.4	92.8	
	1.5	9–11	50.2	73.7	
	1.5	9–19	54.7	37.8	
	1.5	10–11	67.8	78.4	
Average	1.6		73.6	72.9	1.0
Endrin	0.33	8–13	80.1	100	
	.2	8–25	100	100	
	.2	9–11	100	100	
	.125	9–19	98.3	70.3	
	.125	10–11	100	97.6	
Average	.196		95.7	93.6	2.2
Heptachlor	0.33	8–13	72.7	50.0	
	.2	8–25	90.4	50.5	
	.2	9–11	60.4	62.0	
	.2	9–19	47.4	20.8	
	.2	10–11	58.1	50.7	
Average	.23		65.8	46.8	28.9
EPN	0.33	8–13	100	97.1	
	.2	8–25	100	93.4	
	.2	9–11	100	92.1	
	.125	9–19	94.8	56.7	
	.125	10–11	78.8	82.4	
Average	.196		94.7	84.3	11.0
Methyl	0.33	8–13	100	100	
Parathion	.2	8–25	100	100	
	.2	9–11	100	100	
	.125	9–19	100	68.0	
	.125	10–11	97.3	93.0	
Average	.196		99.5	92.2	7.3

[1] Pounds active ingredients per acre.
[2] During the test periods, ave. daily temp.: Max. 92° F., Min. 77° F.; relative humidity: Max 75%, Min. 38%.
[3] Weevils released immediately after treatment into plants receiving no rain.
[4] Weevils released onto plants following application of one-half inch of simulated rain to plants soon after treatment.

toxicity due to the simulated rain.

Experiment 6, (Table 6) was conducted in the laboratory to determine the effect of dew and 0.5 inch of simulated rain on the toxicity of toxaphene, aldrin and dieldrin when used against the boll weevil. Three series of tests were conducted. The treated plants in one series were stored in the laboratory for 24 hours before weevils were released onto them. In another series the treated plants received a simulated heavy dew after being stored in the laboratory for 24 hours. Weevils were then released onto these plants after the dew dried. In still another series the treated plants received 0.5 inch of simulated rain after being stored in the laboratory for 24 hours. Weevils were then released onto these plants after the simulated rain was allowed to dry. The toxicity of toxaphene and dieldrin was not greatly reduced by the dew. One-half inch of simulated rain caused an appreciable reduction in the toxicity of both toxaphene and dieldrin. The toxicity of aldrin was greatly reduced in 24 hours after the treatment, even without the application of dew or rain.

Experiment 7, (Table 7) was conducted in the laboratory to determine the effect of wind on the toxicity of toxaphene, aldrin and dieldrin to the boll weevil. Three

Table 6.—Results of laboratory cage toxicity tests for boll weevil control using insecticides indicated applied as sprays in experiment 6 at College Station, Texas.

Treatment	Dose[1]	Date[2]	Per Cent Mortality			Per Cent Reduction in Toxicity Due to	
			24-Hour Delay[3]	24-Hour Delay Plus Simulated Dew[4]	24-Hour Delay Plus Simulated Rain[5]	Dew	Rain
Toxaphene	1.5	8-16	95.4	94.8	33.3	.6	65.1
Aldrin	0.25	8-16	13.1	0	0	100.0	100.0
Dieldrin	0.25	8-16	100.0	82.7	40.0	17.3	60.0

[1] Pounds active ingredients per acre.
[2] During the test period, ave. daily temp.: Max. 96° F., Min. 83° F.; relative humidity: Max. 81%, Min. 37%.
[3] Treated plants stored inside the laboratory at max. temp. of 96° F. on 8/15. Weevils released onto plants 24 hours after treatment.
[4] Treated plants stored inside the laboratory at max. temp. of 96° F. on 8/15. Weevils released onto plants following application of simulated dew to plants 24 hours after treatment.
[5] Treated plants stored inside the laboratory at max. temp. of 96° F. on 8/15. Weevils released onto plants following application of one-half inch of simulated rain to plants 24 hours after treatment.

series of tests were conducted. One series consisted of releasing weevils onto treated plants immediately following the insecticide applications. Another series consisted of exposing treated plants for 24 hours in the greenhouse, and then releasing weevils onto these plants in the laboratory. The other series consisted of exposing treated plants to simulated wind in the greenhouse for 24 hours, and then releasing weevils onto these plants in the laboratory. The toxicity of toxaphene and dieldrin was not greatly reduced by simulated wind. The toxicity of aldrin was greatly reduced, even in the absence of wind.

Experiment 8, (Table 8) was conducted in the laboratory to determine the effect of high and low temperatures on the toxicity of toxaphene, methyl parathion, EPN, aldrin, dieldrin, heptachlor and endrin to the boll weevil. The plants in this experiment were treated in three series. One series of plants had weevils released onto them immediately following insecticide applications. Another series of plants were stored in an enclosed controlled temperature cabinet at a low temperature (69° F.) for 24 hours after treatment. Weevils were then released onto these plants in the laboratory. The other series of plants were exposed to high temperature (116° F.) in the greenhouse for 24 hours following treatment. Weevils

Table 7.—Results of laboratory cage toxicity tests for boll weevil control using insecticides indicated applied as sprays in experiment 7 at College Station, Texas.

Treatment	Dose[1]	Date[2]	Per Cent Mortality			Per Cent Reduction in Toxicity in	
			Immediate Release[3]	24-Hour Delay[4]	24-Hour Delay Plus Simulated Wind[5]	24 Hours	24 Hours Plus Wind
Toxaphene	1.5	10-8	95.2	89.8	88.8		
	1.5	10-15	85.5	92.3	70.2		
Average	1.5		90.4	91.1	79.5	0	12.1
Aldrin	0.2	10-8	100.0	87.6	51.2		
	.2	10-15	91.0	1.0	32.5		
Average	.2		95.5	44.3	41.9	53.6	56.1
Dieldrin	0.2	10-8	100.0	100.0	100.0		
	.2	10-15	100	91.7	57.8		
Average	.2		100	95.9	78.9	4.1	21.1

[1] Pounds active ingredients per acre.
[2] During the test period, ave. daily temp.: Max. 86° F., Min. 76° F.; relative humidity: Max. 40%, Min. 26%.
[3] Weevils released onto plants immediately after treatment.
[4] Treated plants stored in greenhouse at max. temp. of 98° F. on 10/7 and 110° F. on 10/14. Weevils released onto plants 24 hours after treatment.
[5] Treated plants stored in greenhouse at max. temp. of 98° F. on 10/7 and 110° F. on 10/14. These plants were exposed to simulated wind of 4.9 m.p.h. on 10/7 and 3.8 m.p.h. on 10/14. Weevils released onto plants 24 hours after treatment.

Table 8.—Results of laboratory cage toxicity tests for boll weevil control using insecticides indicated applied as sprays in experiment 8 at College Station, Texas.

Treatment	Dose[1]	Date[2]	Per Cent Mortality			Per Cent Reduction in Toxicity in 24 Hours at	
			Immediate Release[3]	24-Hour Delay at Low Temperatures[4]	24-Hour Delay at High Temperatures[5]	Low Temperatures	High Temperatures
			Test 1				
Toxaphene	1.5	9-26	61.0	82.1	53.4	0	12.5
Methyl Parathion	0.125	9-26	100.0	75.7	82.2	24.3	17.8
EPN	0.125	9-26	95.9	91.8	87.3	4.3	9.0
			Test 2				
Toxaphene	1.5	10-4	37.5	79.2	19.0	0	49.3
Aldrin	0.2	10-4	59.0	37.5	21.3	36.4	63.9
Dieldrin	0.2	10-4	93.0	70.3	56.2	24.4	39.6
			Test 3				
Toxaphene	1.5	10-29	57.8	85.1	55.4	0	4.2
Heptachlor	0.2	10-29	85.2	69.1	56.5	18.9	33.7
Endrin	0.2	10-29	97.1	98.0	100.0	0	0

[1] Pounds active ingredients per acre.
[2] During the test periods, ave. daily temp.: Max. 85° F., Min. 74° F.; relative humidity: Max. 58%, Min. 35%.
[3] Weevils released onto plants immediately after treatment.
[4] Treated plants stored in a cool cabinet at max. temp. of 70° F. on 9/25, 68° F. on 10/3 and 68° F. on 10/28. Weevils released onto plants 24 hours after treatment.
[5] Treated plants stored in the greenhouse at max. temp. of 120° F. on 9/25, 112° F. on 10/3 and 117° F. on 10/28. Weevils released onto plants 24 hours after treatment.

were then released onto these plants in the laboratory. In general the high temperature reduced the toxicity of these insecticides more than did the low temperature. Aldrin and heptachlor showed greater reduction in toxicity than did toxaphene when exposed to the higher temperature. It was noted that the toxicity of the toxaphene-treated plants actually increased when they were placed in the enclosed temperature cabinet with plants treated with the other insecticides. Additional tests indicated that this was not the case when toxaphene treated plants were placed alone in the temperature cabinet. Since most of the plants treated with insecticides other than toxaphene actually lost toxicity at a low temperature in the enclosed cabinet, it was concluded that the vapors given off by these other insecticides either condensed on plant surfaces or were absorbed by the toxaphene treated plants which in turn increased the toxicity of these toxaphene treated plants.

Experiment 9, (Table 9) was conducted in the laboratory to determine the effect of one-half inch of simulated rain on the toxicity of toxaphene, EPN, methyl parathion, malathon and parathion to the cotton leafworm. The procedure used was similar to that described in experiment 5.

Table 9.—Results of laboratory cage toxicity tests for cotton leafworm control using insecticides indicated applied as sprays in experiment 9 at College Station, Texas.

Treatment	Dose[1]	Date[2]	Per Cent Mortality		Per Cent Reduction in Toxicity Due to Rain
			No Rain[3]	Simulated Rain[4]	
Toxaphene	0.125	10–1	0	3.0	
	.125	10–14	91.7	67.6	
Average	.125		45.9	35.3	23.1
EPN	0.01	10–1	97.0	97.0	
	.01	10–14	100	100	
Average	.01		98.5	98.5	0
Methyl	0.005	10–1	85.2	26.6	
Parathion	.005	10–14	77.1	11.8	
Average	.005		81.2	19.2	76.4
Malathon	0.025	10–1	55.6	12.0	
	.025	10–14	100	100	
Average	.025		77.8	56.0	28.0
Parathion	0.005	10–1	91.2	82.9	
	.005	10–14	100	100	
Average	.005		95.6	91.5	4.3

[1] Pounds active ingredients per acre.
[2] During the test periods, ave. daily temp.: Max. 87° F., Min. 76° F.; relative humidity: Max. 59%, Min. 34%
[3] Leafworms released immediately after treatment onto plants receiving no rain.
[4] Leafworms released onto plants following application of one-half inch of simulated rain to plants soon after treatment.

The toxicity of methyl parathion was greatly reduced by 0.5 inch of simulated rain, while the toxicity of toxaphene, malathon, parathion and EPN was not greatly reduced.

Experiment 10, (Table 10), was conducted in the laboratory to determine the effect of 0.5 inch of simulated rain on the toxicity of toxaphene, endrin, isodrin,

Table 10.—Results of laboratory cage toxicity tests for cotton leafworm control using insecticides indicated applied as sprays in experiment 10 at College Station, Texas.

TREATMENT	DOSE[1]	DATE[2]	PER CENT MORTALITY		PER CENT REDUCTION IN TOXICITY DUE TO RAIN
			No Rain[3]	Simulated Rain[4]	
Toxaphene	0.09	1-5	100.0	96.7	
	.04	1-7	100	100	
Average	.065		100	98.4	1.6
Endrin	0.006	1-5	98.5	90.2	
	.003	1-7	93.6	87.5	
Average	.005		96.1	88.9	7.5
Isodrin	0.025	1-5	100.0	92.1	
	.012	1-7	100	90.2	
Average	.019		100	91.2	8.8
Parathion	0.0028	1-5	100.0	92.1	7.9
EPN	.004	1-5	100.0	100.0	0
Malathon	.024	1-5	100.0	54.2	45.8

[1] Pounds active ingredients per acre.
[2] During the test periods, ave. daily temp.: Max. 82° F., Min. 76° F.; relative humidity: Max. 44%, Min. 30%.
[3] Leafworms released immediately after treatment onto plants receiving no rain.
[4] Leafworms released onto plants following application of one-half inch of simulated rain to plants soon after treatment.

parathion, EPN and malathon to the cotton leafworm. The procedure used was similar to that described above. The toxicity of malathon was greatly reduced by one-half inch of simulated rain, while the toxicity of toxaphene, endrin, isodrin, parathion and EPN was not greatly reduced.

Experiment 11 (Table 11) was conducted in the laboratory to determine the effect of simulated wind on the toxicity of toxaphene, EPN, methyl parathion, endrin, dieldrin, γBHC, aldrin, parathion and malathon to the cotton leafworm. The procedure used was similar to that described in experiment 7. In general wind proved to be an important factor in reducing the toxicity of these insecticides when applied at low dosages for cotton leafworm control. For the past several

Table 11.—Results of laboratory cage toxicity tests for cotton leafworm control using insecticides indicated applied as sprays in experiment 11 at College Station, Texas.

TREATMENT	DOSE[1]	DATE[2]	PER CENT MORTALITY			PER CENT REDUCTION IN TOXICITY IN	
			Immediate Release[3]	24-Hour Delay[4]	24-Hour Delay Plus Simulated Wind[5]	24 Hours	24 Hours Plus Wind
			Test 1				
Toxaphene	0.125	10-24	40.1	33.3	12.5	17.0	68.8
EPN	.005	10-24	100	100	100	0	0
Methyl Parathion	.005	10-24	87.2	42.4	17.3	51.4	80.2
			Test 2				
Toxaphene	0.125	10-31	8.3	47.4	0	0	100
Endrin	.01	10-31	69.4	29.7	0	57.2	100
Dieldrin	.05	10-31	7.3	15.9	6.3	0	13.7
			Test 3				
Toxaphene	0.2	11-11	97.0	73.2	40.6	24.5	58.1
Endrin	.01	11-11	86.7	77.7	35.5	10.4	59.1
γBHC	.25	11-11	96.9	73.5	69.5	24.1	28.3
			Test 4				
Toxaphene	0.2	11-13	72.9	77.2	2.4	0	96.7
Aldrin	.25	11-13	83.7	1.3	0	98.4	100
Dieldrin	.05	11-13	42.5	2.8	0	93.4	100
			Test 5				
Toxaphene	0.2	11-18	88.8	86.1	14.8	3.0	83.3
Parathion	.005	11-18	100	100	100	0	0
Malathon	.025	11-18	96.7	26.0	8.2	73.1	91.5

[1] Pounds active ingredients per acre.
[2] During the test periods, ave. daily temp.: Max. 84° F., Min. 76° F.; relative humidity: Max. 54%, Min. 37%.
[3] Leafworms released onto plants immediately after treatment.
[4] Treated plants stored in greenhouse at max. temp. of 106° F. on Oct. 23, 112° F. on Oct. 30, 101° F. on Nov. 10, 106° F. on Nov. 12 and 94° F. on Nov. 17. Leafworms released onto plants 24 hours after treatment.
[5] Treated plants stored in greenhouse at max. temp. of 106° F. on Oct. 23, 112° F. on Oct. 30, 101° F. on Nov. 10, 106° F. on Nov. 12 and 94° F. on Nov. 17. These plants were exposed to simulated wind of 4.0 m.p.h. on Oct. 23, 4.6 m.p.h. on Oct. 30, 3.7 m.p.h. on Nov. 10, 4.7 m.p.h. on Nov. 12 and 12.4 m.p.h. on Nov. 17. Leafworms released on plants 24 hours after treatment.

Table 12.—Results of laboratory cage toxicity tests for salt-marsh caterpillar control, using insecticides indicated applied as sprays in experiment 12 at College Station, Texas.

Treatment	Dose[1]	Date[2]	Per Cent Mortality		Per Cent Reduction in Toxicity Due to Rain
			No Rain[3]	Simulated Rain[4]	
Toxaphene	0.35	2-5	87.0	82.6	
	.5	2-9	65.1	65.7	
Average	.43		76.1	74.2	2.5
TM-1	0.35	2-5	83.0	91.1	
	.5	2-9	82.0	95.3	
Average	.43		82.5	93.2	0
Heptachlor	0.2	2-5	60.0	0	
	.6	2-9	93.2	39.7	
Average	.4		76.6	19.9	74.0
Methyl Parathion	0.1	2-9	26.2		
EPN	0.2	2-9	14.6		
Malathon	0.2	2-9	11.4		

[1] Pounds active ingredients per acre.
[2] During the test periods, ave. daily temp.: Max. 85° F., Min. 79° F.; relative humidity: Max. 48%, Min. 33%.
[3] Caterpillars released immediately after treatment onto plants receiving no rain.
[4] Caterpillars released onto plants following application of one-half inch of simulated rain to plants soon after treatment.

years toxaphene has proved to be one of the most stable insecticides when exposed to the various climatic conditions. Results of this experiment indicate that wind reduces the toxicity of toxaphene more than any other climatic factor studied.

Experiment 12, (Table 12), was conducted in the laboratory to determine the effect of 0.5 inch of simulated rain on the toxicity of toxaphene, TM-1 and heptachlor to the salt-marsh caterpillar. The procedure used was similar to that described in experiment 5. The toxicity of heptachlor was greatly reduced by one-half inch of simulated rain. Rain had little or no effect on the toxicity of toxaphene and TM-1.

SUMMARY AND CONCLUSIONS.—Results of laboratory and field cage tests for boll weevil control indicate that it required from 2 to 3 times more of the various insecticides to kill comparable percentages of weevils in the field than in the laboratory. This ratio in effectiveness was less this year than last year, due to different environmental conditions. The temperatures were higher and the percentages of relative humidity were lower in 1951 than in 1952. Such climatic factors as high temperatures, wider ranges in relative humidity, sunlight, dew and wind were important in reducing the toxicity of the various insecticides in the field. When exposed to normal climatic conditions, both in the field and outside of the laboratory, dieldrin retained its toxicity for boll weevils during a longer period of time than did any of the other insecticides used. Toxaphene, EPM and endrin retained their toxicity for weevils under these various climatic conditions for a longer period of time than did the other remaining insecticides tested. The toxicity of γBHC, aldrin, heptachlor and methyl parathion was greatly reduced by the climatic factors mentioned above. Aldrin and heptachlor showed an appreciable loss in toxicity, even when protected from normal climatic conditions inside of the laboratory.

It required from 2 to 3 times as much of the various insecticides to kill comparable percentages of boll weevils late in the season as compared to early in the season. Since the climatic conditions did not vary sufficiently to account for such a difference, it is assumed that factors involving the physiology of the weevil are responsible for such varying susceptibility. This seasonal resistance did not reach as high a peak this year as it has in some previous years.

There was no appreciable reduction in the toxicity of toxaphene, EPN, methyl parathion and endrin to boll weevils following 0.5 inch of simulated rain applied to plants soon after treatment. The toxicity of heptachlor was reduced appreciably by the simulated rain. An application of simulated dew to plants 24 hours after treatment caused little or no reduction in the toxicity of toxaphene and dieldrin to weevils. However, 0.5 inch of simulated rain applied 24 hours after treatment caused an appreciable reduction in the toxicity of toxaphene and dieldrin. The toxicity of aldrin was greatly reduced in 24 hours, even without the application of dew or rain. Rain when applied 24 hours following the insecticidal treatment reduced the toxicity of the materials more than did rain applied soon after the treatment. Aldrin and heptachlor showed a greater reduction in toxicity due to the effects of rain than did any of the other insecticides studied for boll weevil control. These sprays were either washed off the plants or the rain caused a breakdown of the chemical.

Dew alone did not greatly reduce the toxicity of the insecticides. However, the effects of dew may have a relation to the action of other climatic factors such as sunlight, wind and rain.

In general high temperature (116° F.) reduced the toxicity of toxaphene, EPN, aldrin, dieldrin and heptachlor when used to control the boll weevil more than did low temperature (68° F.). Aldrin and heptachlor showed greater reduction in toxicity than did the other insecticides when exposed to the higher temperature.

The toxicity of toxaphene and dieldrin when used against the boll weevil was not greatly reduced by a 24-hour exposure of treated plants to simulated wind (4.7 m.p.h.). The toxicity of aldrin was greatly reduced during the 24-hour period, even in the absence of wind. Simulated wind (5.9 m.p.h.) proved to be effective in reducing the toxicity of toxaphene, methyl parathion, endrin, dieldrin, γBHC, aldrin and malathon when applied for cotton leafworm control. Since the beginning of this study, the toxicity of toxaphene has not been greatly affected when exposed to the various climatic conditions. Results of the leafworm experiment indicate that wind reduced the toxicity of toxaphene more than any other climatic factor studied.

One-half inch of simulated rain applied soon after the insecticidal treatment caused little or no reduction in the toxicity of toxaphene, EPN, parathion, endrin and isodrin when used to control the cotton leafworm. The toxicity of methyl parathion and malathon was greatly reduced by simulated rain. The toxicity of heptachlor, when used against the salt-marsh caterpillar, was greatly reduced by 0.5 inch of simulated rain. There was little or no reduction in the toxicity of toxaphene and TM-1 following rain. Excessive amounts of insecticides are usually applied in the field for control of other cotton insects; and those amounts should give good control of the leafworm even following 0.5 inch of rain.

Toxicity of insecticides has been found to vary with the species of insects used, perhaps due to habits and feeding mechanisms of the insects. The contact and stomach activity of the insecticides may also vary with the test species. Worms consume large amounts of foliage, while weevils consume comparatively little; therefore the worm tests are generally considered to be more critical.

It should be pointed out that certain insecticides, although lacking in residual effectiveness, are highly effective under normal weather conditions in controlling an initial infestation of insects. Due to the lack of residual effectiveness of certain insecticides, insects migrating into a field or hatching 1 or 2 days after application are little affected. Under extremely severe weather conditions such insecticides may fail to give satisfactory control even immediately after treatment.

Furthermore some insecticides remain toxic for 1 or 2 days after application when subjected to the normal climatic factors, but they are slow in action. When these materials are used plant damage may occur before the insects are killed. Other insecticides have the quality of being quick-acting and also possess long residual toxicity.

Under normal climatic conditions, certain insecticides loose their toxicity rapidly regardless of the intensity of these conditions. Extremes in the various climatic factors only tend to accelerate the process of the loss in toxicity. Other insecticides appear to retain their toxicity during long periods of time and only the greatest extremes in the climatic conditions cause loss in toxicity.

A problem exists in the study of organic insecticides under controlled conditions. In order to produce artificially controlled weather conditions it is necessary to place treated plants in some type of enclosed cabinet. When these treated plants are enclosed, some of the insecticides vaporize which in turn contaminates the cabinet and may alter the results of the experiment. This phenomenon is one of the chief factors limiting the study of these insecticides.

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The Toxicity of Certain Insecticides to the Overwintered Boll Weevil[1]

W. J. Mistric, Jr. and J. C. Gaines, *Texas Agricultural Experiment Station*

There appears to be little published evidence with regard to the toxicity of insecticides on the overwintered boll weevil. Overwintered weevils are difficult to collect in sufficient quantities to include in replicated tests. This difficulty in obtaining weevils is probably responsible for the lack of experimental work.

In field cage tests conducted at this Station, Gaines & Mistric (1952) found that a comparatively low dosage of toxaphene was required to kill overwintered weevils early in June. It required nearly 3 times the dosage to kill a comparable percentage of weevils reared from squares, and a still higher dosage was required to kill comparable percentages of boll-reared weevils and field collected weevils later in the season. It was thought that the food upon which the weevils developed may have been partially responsible for this increasing resistance to toxaphene as the season progressed. The depletion of food in the overwintered weevils may have been responsible for their lowered resistance to toxaphene.

Gaines & Mistric (1951, 1952) and Mistric & Gaines (1953) showed that considerably higher dosages of certain insecticides were required to give a kill of weevils in field cages comparable to that obtained in the laboratory. Such climatic factors as high temperature, wider ranges in relative humidity, wind, sunlight and dew were important in reducing the toxicity of the various insecticides in the field. It was also found that considerably higher dosages of certain insecticides were required to give a kill of field collected weevils late in September and October comparable to that obtained in August. Since the climatic conditions did not vary sufficiently to account for such a difference in control, it was assumed that factors other than environmental greatly affected the toxicity of the insecticides. Probably the physiology of the weevil itself was involved.

Results of field experiments by many workers indicate that low dosages of the organic insecticides are required to control overwintered weevils. Later in the season comparatively high dosages of these materials are required for weevil control.

The tests herein reported were conducted at College Station, Texas during 1952 to obtain information regarding the effectiveness of certain organic insecticides when used for control of the overwintered boll weevil.

Methods and Materials.—All of the organic insecticides currently recommended for early season control of the boll weevil in Texas were used in this study. These insecticides included toxaphene, aldrin dieldrin, heptachlor and γ BHC. All of the materials were applied as emulsion sprays at normal dosages.

Overwintered boll weevils were available in sufficient quantities to conduct both laboratory and field cage tests. The weevils were collected from seedling cotton in the field shortly before the beginning of each test.

A hygrothermograph was used to record the temperature and relative humidity. The percentage mortalities were calculated by Abbott's formula. The dosages are shown as pounds of active ingredients per acre. Comparable untreated checks were used in each test. Survival records were made over a period of 5 days following the release of weevils onto treated plants.

In the laboratory cage tests the sprays were applied to potted cotton plants. A pressure of 60 pounds per square inch was used to dispense the spray through two hollow cone nozzles at the rate of 5 gallons per acre. The plants were rotated through the spray as described in previous papers from this station. The percentage mortality shown for each dosage represents the average of four replicate cages using about 15 weevils per cage.

In the cage tests conducted in the field, the sprays were applied to individual cotton plants enclosed in a cylinder. A pressure of 60 pounds per square inch was used with a solid cone nozzle to dis-

[1] Technical Contribution No. 1716, Texas Agricultural Experiment Station in Cooperation with the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

pense the spray at the rate of 7.5 gallons per acre. The percentage mortality shown for each dosage represents the average of four replicate cages using about 25 weevils per cage.

RESULTS.—Experiment 1 shown in table 1 was conducted in the laboratory to determine the toxicity of toxaphene, aldrin, dieldrin, heptachlor and γ BHC when used for the control of the overwintered boll weevil. The weevils were caged on treated plants immediately following the insecticide applications. The entire experiment was conducted inside of the laboratory protected from normal climatic conditions. The average daily temperatures in the laboratory during the test periods ranged from 77° to 87° F., and the average daily relative humidities ranged from 52 to 85 per cent. At the dosages tested, all of the insecticides used were effective in controlling the overwintered boll weevil with the exception of heptachlor. Apparently a slightly higher dosage of heptachlor is needed to control overwintered weevils.

Table 1.—Results of laboratory cage toxicity tests for control of the overwintered boll weevil using the insecticides indicated applied as sprays in experiment 1 at College Station, Texas.

TREATMENT	DOSE[1]	DATE	TOTAL NO. OF WEEVILS	PER CENT MORTALITY
Toxaphene	1.5	5–30	59	93.6
	1.5	6–7	49	71.9
	1.5	6–11	60	100
Average	1.5		168	88.5
Aldrin	.25	5–30	61	100
	.25	6–7	59	100
	.25	6–11	56	69.6
Average	.25		176	89.9
Dieldrin	.25	5–30	58	93.5
	.25	6–7	52	100
	.25	6–11	60	100
Average	.25		170	97.8
Heptachlor	.25	5–30	60	62.0
	.25	6–7	56	75.5
	.25	6–11	55	0
Average	.25		171	45.8
γ BHC	.2	5–30	60	87.4
	.2	6–7	56	100
	.2	6–11	48	39.1
Average	.2		164	75.5

[1] Pounds active ingredients per acre.

Experiment 2 shown in table 2 was conducted in the field to determine the toxicity of toxaphene, aldrin, dieldrin, heptachlor and γ BHC when used for control of the overwintered boll weevil. The weevils were caged on treated plants immediately following insecticide applications. Normal climatic conditions prevailed throughout the experiment. The average daily temperatures during the test periods ranged from 74° to 95° F., and the average daily relative humidities ranged from 40 to 87 per cent. There were heavy dews every morning during the test periods and the days were cloudy. The average daily wind movement during the test periods was 6.3 miles per hour. Dieldrin was the only insecticide tested that was effective in controlling the overwintered weevil under these conditions. The toxicity of aldrin and toxaphene was greatly reduced, while heptachlor and γ BHC were infective.

Table 2.—Results of field cage toxicity tests for control of the over wintered boll weevil using the insecticides indicated applied as sprays in experiment 2 at College Station, Texas.

TREATMENT	DOSE[1]	DATE	TOTAL NO. OF WEEVILS	PER CENT MORTALITY
Toxaphene	1.5	6–19	99	50.5
	1.5	6–20	97	14.1
Average	1.5		196	32.3
Aldrin	.25	6–19	88	70.5
	.25	6–20	94	18.7
Average	.25		182	44.6
Dieldrin	.25	6–19	99	84.0
	.25	6–20	99	98.9
Average	.25		198	91.5
Heptachlor	.25	6–19	99	30.1
	.25	6–20	93	5.5
Average	.25		192	17.8
γ BHC	.2	6–19	100	39.5
	.2	6–20	99	0.0
Average	.2		199	19.8

[1] Pounds active ingredients per acre.

DISCUSSION.—The field cage tests were conducted one week following the laboratory tests. In tests conducted during this interval, toxaphene at 1.5 pounds per acre gave 100 per cent control of the overwintered weevil in the laboratory, whereas on the same date and at the same dosage toxaphene gave only 41 per cent control in the field cage test.

A considerably higher percentage of weevils survived in the checks in the field than did in the laboratory. The warm, moist, cloudy conditions in the field were probably more conducive to weevil sur-

vival than were the conditions in the laboratory. The occurrence of these weather conditions favorable for the life of the boll weevil may be related to an increased resistance of the weevil to insecticides in the field.

It is known from previous work that from 2 to 4 times as much of the various insecticides is required to give mortalities of weevils in the field cages comparable to that obtained in the laboratory. It is recognized that the normal climatic conditions in the field reduce the effectiveness of the insecticides, thus requiring greater dosages. In light of these recent observations, the relatively high wind, heavy dews, and high temperature encountered in the field were important factors in reducing the toxicity of toxaphene, aldrin, heptachlor and γ BHC. Dieldrin was the only insecticide tested that gave effective control of the overwintered weevil in the field cage tests.

All of the materials used gave effective control of the overwintered weevil in the laboratory tests with the exception of heptachlor. Apparently a higher dosage of heptachlor than that used is needed to control the weevil. The laboratory experiment was conducted inside of the laboratory protected from the climatic conditions that prevailed in the field.

In view of previous work conducted at this laboratory and of favorable results obtained by many workers with the early season control of weevils in the field, it is generally agreed that overwintered weevils can be satisfactorily controlled with relatively low dosages of the various insecticides due to the lowered resistance of the weevils. However, as shown in this study, under certain conditions favorable to the boll weevil and unfavorable for the insecticides, higher dosages of insecticides may be needed in such instances to obtain effective control.

SUMMARY.—Laboratory and field cage tests were conducted to determine the effectiveness of toxaphene, aldrin, dieldrin, heptachlor and γ BHC when used to control the overwintered boll weevil. In the laboratory tests all of these insecticides gave effective control of the weevil with the exception of heptachlor which was used at a low dosage. In the field cage tests dieldrin was the only insecticide tested that gave effective control of the overwintered weevil. The toxicity of toxaphene and aldrin was greatly reduced, while heptachlor and γ BHC proved ineffective.

It is generally agreed that overwintered weevils, due to their lowered resistance, can be satisfactorily controlled early in the season with relatively low dosages of certain organic insecticides. However, the results of this study indicate that when certain climatic conditions are favorable to the boll weevil and unfavorable for the insecticides higher dosages of the insecticides may be needed to obtain effective control of the overwintered boll weevil.

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EXPEDITION TO BAHAMA ISLANDS

The Van Voast-American Museum of Natural History, Bahama Islands Expedition sailed from Myrtle Beach, South Carolina, on December 15, 1952, for the Bahama Islands where for six months the expedition will collect and preserve biological material, with particular emphasis on insects, spiders, reptiles and amphibia. If time permits sea urchins and land snails will be collected. The staff consisted of Horace S. Van Voast, Jr., president of the Van Voast Insurance Agency, Schenectady, New York, co-owner and captain of the 43-foot auxiliary schooner, "White Wing"; John C. Pallister, research associate in the Museum's department of insects and spiders; Ellis B. Hayden, Jr., a graduate student in entomology at the University of Kansas, and George B. Rabb, a graduate student at the University of Michigan.

SCIENTIFIC NOTES

Control of Rhododendron Whitefly, Woolly Larch Aphid, Rose Scale and Southern Red Mite[1]

T. W. KERR JR., *University of Rhode Island, Kingston*

The comparative effectiveness and timing of application of various insecticides for control of 19 species of insects attacking ornamental trees and shrubs has been reported previously (Kerr 1950, 1951, 1952). This report is a continuation of those investigations. It concerns field experiments undertaken in 1952 involving the rhododendrom whitefly, *Dialeurodes chittendeni* Laing, the rose scale, *Aulacaspis rosae* (Bouche), the woolly larch aphid, *Chermes strobilobius* Kltb. and the southern red mite, *Paratetranychus ilicis* (McG.).

MATERIALS.—The details relative to the materials, concentrations and combinations used in each experiment are given in tables 1 to 4. The chlorinated hydrocarbon insecticides were wettable powders and included 75 per cent DDT and 25 per cent lindane. Nicotine sulfate was employed as the conventional 40 per cent solution. Commercially available acaricidal preparations of 50 per cent p-chlorophenyl p-chlorobenzene sulfonate[2] and 15 per cent chloroethyl butylphenoxy methyl ethyl sulfite[3] were used as wettable powders. Additional wetting, spreading and sticking agent,[4] at the rate of 4 ounces in 100 gallons of water, was used with the chlorinated hydrocarbon insecticides, the two acaricides and with nicotine sulfate in each experiment.

Table 1.—Control of the rhododendron whitefly with various insecticides. Coventry, R. I. June–October, 1952.

MATERIAL	AMOUNT IN 100 GAL. WATER	APPLICATIONS	LARVAE PER 100 SQ. IN. OF LEAF	CONTROL
		No.	No.	%
DDT 75 per cent W	1.33 lb.	3	2	99.9
		2	46	97.8
	0.67 lb.	3	4	99.8
		2	12	99.4
Lindane 25 per cent W	1 lb.	3	54	97.5
		2	229	89.3
	0.5 lb.	3	1070	49.9
		2	386	81.9
Nicotine sulfate 40 per cent	1 pt.	3	45	97.9
		2	173	91.9
Check			2136	

The petroleum oil used was a "superior" type miscible dormant spray oil, the specifications for which were outlined by Chapman & Pearce (1949). All materials were applied as sprays with a conventional power-operated, hydraulic sprayer and spray gun.

RHODODENDRON WHITEFLY.—This insect is an occasional pest of rhododendron in Rhode Island. Its seasonal history is similar to that outlined for Washington state by Latta (1937). In 1952, the first adults were observed on May 27 when the new leaves on some of the rhododendrons had begun to unfurl. The peak of emergence was reached on June 5. The adult population had begun to decline by June 16, and on June 24 none could be found, even on the untreated plants in the experimental area. Hatching of the eggs began on June 23 and by July 7, 98 per cent of the larvae had appeared.

The experiment was conducted on rhododendron plants, *Rhododendron catawbiense* Mich., averaging 3 feet in height and heavily infested with whiteflies. Each treatment was applied to 6 plants on June 5 and June 23 at the rates indicated in table 1. On July 7, three of the plants in each treatment received a third application of spray. Data on control were obtained during the period of October 16 to 22, when an average of 118 square inches of the under sides of the basal leaves of the current year from each plant in all treatments were examined for live young. At that time, most of the young surviving the treatments were third instar. The results presented in table 1 show that all of the insecticides were effective for controlling the insect. DDT was slightly more effective than lindane or nicotine sulfate, particularly at the lower concentration applied twice. Records obtained during oviposition and hatching of the eggs showed that the chlorinated hydrocarbon insecticides were effective mainly by preventing oviposition. Most of the young hatching from the few eggs deposited in these treatments were killed by residual deposits of the insecticides. While nicotine sulfate prevented some oviposition, it was effective principally as an ovicide.

ROSE SCALE.—This scale has been found in various local areas during most seasons. It has been abundant primarily on rugose rose, *Rosa rugosa* Thumb. In 1952 in the area in which the experiment was conducted, no eggs were found among the partly-grown and full-grown females until May 22. Egg production continued throughout June, and as late as July 16 eggs were being produced by the overwintered female scales. Nymphs of the first generation were seen as early as June 12. The experiment was conducted on two adjoining hedges of rugose rose each of which was 3 feet high, 6 feet deep and 80 feet long. The hedges were divided into 24 equal areas to provide for five insecticide treatments and an unsprayed check, each replicated 4 times. On June 20 the first spray was applied. On July 1 and July 17 certain of the areas received second and third applications. On all these dates, adult females were producing eggs, and newly hatched nymphs

[1] Contribution No. 812 from the Rhode Island Agricultural Experiment Station, Kingston, R. I.
[2] Ovotran, Dow Chemical Company.
[3] Aramite, U. S. Rubber Co.
[4] Triton B-1956, a modified phthalic glycerol alkyd resin.

Table 2.—Control of female rose scale with various insecticides. Westerly, R. I. June–August, 1952.

MATERIAL	AMOUNT IN 100 GAL. WATER	APPLICATIONS	SCALES PER CM.[2] OF BARK	CONTROL
		No.	No.	%
Nicotine sulfate 40 per cent	1 qt.	1	1.37	44.3
		2	1.01	58.8
		3	.40	83.7
DDT 75 per cent W	2.67 lb.	2	1.41	42.7
		3	1.04	57.6
Check			2.45	

were found emerging from beneath the female scales. Data on control were obtained during the period of August 15 to 18 when 125 square centimeters of bark from year-old canes in each replicate of the treatments were examined for live female scale. The results presented in table 2 show that nicotine sulfate was considerably more effective than DDT for controlling the insect. Even so, three applications of nicotine sulfate resulted in only 83.7 per cent control of the female scale.

WOOLLY LARCH APHID.—For several years this insect has been a serious pest of American larch, *Larix laricina* Koch, in Rhode Island. The experiment was conducted on trees averaging 40 feet in height and heavily infested with aphids. One-half of a tree divided perpendicularly was used for each treatment. When the petroleum oil and petroleum oil plus nicotine sulfate treatments were applied on April 11 none of the adult sistens had produced eggs. At that time, new leaves on most of the fascicles averaged 4 millimeters in length. The first eggs produced were observed on April 14. These began to hatch on May 2. The verdant sprays of lindane and nicotine sulfate were applied on May 27. Data on control were obtained on May 29 and 30 when 150 fascicles from random twigs in each treatment were examined for live aphids. Results given in table 3 show that all of the insecticides were effective for controlling the woolly larch aphids. The dormant sprays of petroleum oil and petroleum oil plus nicotine sulfate (Herrick 1935) were more effective than lindane or nicotine sulfate applied as verdant sprays. Neither of the latter two insecticides was effective as an ovicide. A trace of injury to the newly developing leaves occurred as a result of both dormant treatments.

SOUTHERN RED MITE.—In a previous publication (Kerr 1950) it was shown that two sprays containing DDT were effective for controlling the holly leaf miner, *Phytomyza ilicis* (Curt.) on American holly, *Ilex opaca*. Subsequent to 1949, the holly in the experimental area were sprayed each year with DDT to control the insect. The population of southern red mite on the leaves was observed to increase and become more widespread each year. Accordingly, in 1952 certain of the more heavily infested trees were used in an effort to solve the problem. The experiment was conducted on trees averaging 9.7 feet in height. Three trees were used for each treatment. On May 23, when the first spray was applied, the foliage of the previous season was infested with an average of 12.7 mites per leaf. At that time, the new growth averaged 1 inch in length. On June 2, a second spray was applied.

Table 3.—Control of the woolly larch aphid with various insecticides. North Kingstown, R. I. April–May, 1952.

MATERIAL	AMOUNT IN 100 GAL. WATER	APHIDS PER FASCICLE	PER CENT CONTROL
Petroleum oil	3 gal.	0.0	100.0
Petroleum oil+ Nicotine sulfate 40 per cent	2 gal. 1 pt.	0.0	100.0
Lindane 25 per cent W	1 lb.	1.6	91.9
	0.5 lb.	2.2	89.4
Nicotine sulfate 40 per cent	1 pt.	.8	95.9
Check		20.3	

Data on control were obtained on July 2 and September 29, 30 when 25 random leaves from the current year's production on each tree were examined for mites. It was planned to obtain additional data on August 2, but an examination at that time revealed that almost no mites were present. On October 23, the foliage of the current year was examined for mite injury. The results given in table 4 show that the mite population of the check treatment declined throughout the season, while injury to the leaves was slight. In the DDT treatment the mite population was greater than all other treatments in July, but was at a low level in September with a moderate amount of leaf injury. One application of either of the two acaricides combined with DDT resulted in the largest mite populations and severe leaf injury at the end of the season. Two applications of acaricide with DDT resulted in fewer mites in July than in September. There was no mite injury to the foliage of the DDT plus chloroethyl butylphenoxy methyl ethyl sulfite treatment, while that in which p-chlorophenyl p-chlorobenzenesulfonate was combined with DDT showed a trace of mite injury. Both one and two applications of p-chlorophenyl p-chlorobenzenesulfonate resulted in chemical injury to holly foliage. The population of holly leaf miner had been reduced to a minimum by applications of DDT in 1950 and 1951. Therefore, no data on its control with the materials tested in table 4 were obtained.

SUMMARY.—During 1952 the relative effectiveness of several old and new materials, and the timing of

Table 4.—Southern red mite populations on American holly sprayed with various materials. Wakefield, R. I. May–September, 1952.

MATERIAL	POUNDS IN 100 GAL. WATER	APPLICATIONS	MITES PER LEAF ON 7-2-52	MITES PER LEAF ON 9-30-52
		No.	No.	No.
DDT 75 per cent W	1.33	2	9.40	1.24
DDT 75 per cent W+chloroethyl butylphenoxy methyl ethyl sulfite 15 per cent W	1.33 1.5	1[1]	.24	50.44
		2	.12	.95
DDT 75 per cent W+p-chlorophenyl p-chlorobenzene sulfonate 50 per cent W	1.33 1	1[1]	.04	22.35
		2	.02	3.15
Check			3.90	.76

[1] These treatments received a second spray containing 1.33 lb. 75% W DDT in 100 gal. water.

such pesticidal applications, was investigated in field tests involving three insects and one mite attacking ornamental trees and shrubs.

DDT was slightly more effective than lindane or nicotine sulfate for controlling the rhododendron whitefly. Two applications, each containing two-thirds of a pound of wettable 75 per cent DDT in 100 gallons of water, the first at the peak of adult emergence in early June and the second 18 days later, provided the best control. The chlorinated hydrocarbon insecticides prevented oviposition while nicotine sulfate was effective principally as an ovicide.

Nicotine sulfate was effective and DDT ineffective for controlling the rose scale. Even so, three sprays, each containing 1 quart of 40 per cent nicotine sulfate in 100 gallons of water, applied 8, 19, and 35 days after the eggs began to hatch in mid-June, resulted in only 83.7 per cent control of the female scale.

Both 3 per cent petroleum oil and 2 per cent petroleum oil plus 1 pint of nicotine sulfate applied as dormant sprays in early April were 100 per cent effective in controlling the overwintering form of the woolly larch aphid. Verdant sprays of lindane and of nicotine sulfate applied in late May were slightly less effective than the dormant sprays primarily because both materials were not ovicidal.

Previous investigations have shown that two sprays containing DDT were effective for controlling the holly leaf miner on American holly. However, the repeated use of DDT has promoted the increase of southern red mite. Both chloroethyl butylphenoxy methyl ethyl sulfite and p-chlorophenyl p-chlorobenzenesulfonate were effective in reducing the mite population when included with DDT in both sprays, but resulted in an increase in the mite population when included in the first spray only. Phytotoxicity to holly foliage was experienced with p-chlorophenyl p-chlorobenzenesulfonate.

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Gypsy Moth Sex Attractant from Europe for Use in Trapping Program

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Annual surveys in which traps baited with sex attractant are used to capture male moths constitute an important part of the campaign by the Bureau of Entomology and Plant Quarantine against the gypsy moth, *Porthetria dispar* (L.). From 5 to 7 million acres have been trapped each season to obtain a picture of the conditions existing along the periphery of known infestations and to check on the effectiveness of recent spraying work. The entire control program of this Bureau and cooperating state agencies is dependent upon trapping surveys. A highly efficient trapping device has been devised through experimentation. The traps are baited with a sex attractant derived from virgin female moths. This attractant is obtained by collecting female pupae, rearing adult moths in the laboratory, and clipping the two posterior abdominal segments into benzene.

In order to collect large numbers of healthy female gypsy moth pupae, widespread areas of heavy infestation are necessary. Usually within or adjacent to such areas there are places each season where the insect thrives relatively free from disease and parasitism. The Cape Cod section of Massachusetts provided such an area for many years. Although infestations have fluctuated greatly during recent years over most of New England, on Cape Cod the infestation remained heavy with resultant defoliation every year. Accordingly, most of the sex attractant used in the trapping programs was obtained there until the large-scale aerial spray program in 1949 and 1950 eliminated the gypsy moth from that area. A collection and rearing program was attempted in Norfolk County, Massachusetts, in 1950, but disease and parasitism were so widespread that only a small amount of attractant material was obtained. At that time gypsy moth infestation was at a low ebb over most of New England, and it appeared that good collection areas would not be available in the summer of 1951.

The abdominal tips of 15 female moths are required to furnish the attractant material for baiting one trap. Trapping programs in recent years called for the use of about 16,000 traps, requiring 240,000 processed tips. As the season of 1951 approached, the stockpile of material was sufficient for only two full-scale trapping programs. With no dependable domestic supply in sight, it was decided to turn to Europe or North Africa. Attractant material from the French Morocco had been tested during the summer of 1948 and proved fully as attractive as domestic material. There appeared to be no reason why material from Europe should prove other than attractive.

Accordingly, plans were made to send abroad an employee of the Bureau to make a survey of several countries in southern Europe where gypsy moth infestations have been known to exist in recent years. Provided a suitable area could be located, it was decided to set up a collection and rearing program if the many attendant details of such a program could be worked out. Contacts were made directly from the home office and through a resident entomologist of the Bureau near Paris with well-known entomologists and insect collectors in the several countries to be visited. Inasmuch as it was not known where the collection center would be established, it was

decided to assemble all necessary supplies at the Bureau laboratory near Paris. Certain supplies were shipped by boat to Paris, among them thiophene-free benzene, which is very difficult to obtain in Europe.

I was selected for this assignment and left for Paris in February 1951. I soon learned that no large areas of known gypsy moth infestation existed in France and spent no time on field trips there.

In Spain, however, outbreaks have occurred over large areas periodically for many years. From 1922 through 1927 employees of the Bureau were engaged in making collections of gypsy moth parasites in Spain and other central and southern European countries. I therefore went to Madrid, where I met an entomologist still in the Spanish forest service who had been associated with our entomologists in the earlier parasite work. This gentleman accompanied me on a survey of the areas in Spain where infestations have been known to exist in recent years, but after a month we found no infested area of sufficient size and intensity to provide good collecting conditions. While in Spain I hired a young man as interpreter who spoke several European languages fluently and who subsequently became my field assistant and accompanied me throughout my assignment in Europe.

I next made a visit to Portugal. Following a conference with forestry officials in Lisbon, I made a trip to an area about 40 miles south of that city where a heavy gypsy moth infestation was known to exist. There I found a large area of infestation of sufficient intensity to provide good collecting conditions if healthy material could be obtained. After a hasty survey of this area I made a trip to Italy to check on reports of infestations near Naples, in case late-season developments of disease or parasitism wiped out the area south of Lisbon. However, no infestation of sufficient size to be of any use was located in Italy.

Upon returning to Portugal I made a more careful survey of the infested area and found a heavy infestation centering around the Estate village of Palma which covered about 50,000 acres. North of this main infestation were several much smaller areas that could be used for small-scale collections.

There were then three major problems to be considered—labor, a centrally located laboratory, and transportation. It was evident from the first contact that hearty cooperation could be depended on from the Portuguese Government as represented by the Forest Services. They indicated that efforts would be made to provide some means of transportation if other facilities could not be found. A meeting was arranged with the owners of the Estate of Palma. They proved to be deeply interested in our project and offered to assist in every was possible. A large building at Palma was made available for a collection and rearing center, and arrangements were made to hire employees of the Estate for collecting and laboratory work. Living quarters were also provided for me and my field assistant for the duration of the project.

As soon as a location for the laboratory was established, arrangements were made for shipping supplies from Paris. The thiophene-free benzene had arrived at Le Havre, France, but coastal shipping and import regulations made reshipment to Portugal a very difficult problem. Through the help of the United States Embassy these hurdles were cleared, and the benzene was delivered at Palma just four days before it was needed.

Rearing trays of various sizes were hastily constructed to fit into every available space in the laboratory room. Sufficient space was provided to handle 500,000 pupae. After female moths emerge from rearing trays, they are placed in boxes and held in a cool room for 24 hours, which has been determined as the optimum period required to develop the sex attractant. The makeshift laboratory worked out very well, and mortality of moths from handling was low. Young women were used for laboratory work inasmuch as men with sufficient education to maintain necessary records were not available.

Frequent field surveys were made during May to keep a constant check on larval populations and the incidence of disease and parasitism. In Portugal pupae develop late in May, and the best collecting period is usually the first three weeks in June. This is about one month earlier than the collecting season in the northeastern United States. It became evident near the end of May that total defoliation would occur over much of the infested area. This greatly reduced the available collecting zones, as healthy pupae can seldom be obtained within areas of complete defoliation because of starvation, disease, and parasitism.

Field collections of pupae were started early in June. Seven supervisory employees of the Portuguese Forest Services were transferred to us for the duration of the project. Our transportation problems were solved when we were loaned a pick-up truck and a jeep, the only cost to us being for operation. The Portuguese Government paid the salaries of all their regular employees while they were detailed to our work; we paid only their expenses away from the station and for overtime work.

Collection of gypsy moth pupae is much more difficult in Portugal than on Cape Cod. The most important host trees in Portugal are cork oaks. These trees do not grow to a great height, averaging from 30 to 40 feet at maturity. The limbs have a wide spread, and the trees look like large old apple trees. However, they are usually pruned to a height of 8 or 10 feet so that the cork can be easily stripped from the main trunk. Inasmuch as the main trunks are smooth after stripping, few pupae develop there. Since few of the limbs can be reached from the ground, ladders must be used for much of the collecting. Over 75 per cent of all pupae obtained were collected by that method, which is slow and tedious. A collection of 2,000 pupae by one man in a day was very high, whereas in this country daily collections of 6,000 pupae are common. Nevertheless good results were obtained.

An incentive method of compensation was used in paying the laborers hired from Palma. It was based on quality of material as well as quantity, and worked out very well. Although uneducated and generally unable to read or write, the men were highly intelligent and learned quickly. They proved to be excellent workers, anxious to do good work, and willing to put in as many hours daily as necessary. During the collecting season work was carried on 7 days a week. The price of labor, including bonus payments, averaged about $1.75 per day in our currency. The young women hired for laboratory work were paid at nearly twice this rate. Our peak personnel load was 30 persons, including 21 in the the field, 8 in the rearing laboratory, and an over-all supervisor.

During the 1951 season of nearly 4 weeks, a total of 472,000 gypsy moth pupae were collected, from

which 172,500 female tips, or 37 per cent, were obtained. The average cost per tip was 5 cents, based on the entire cost of the work, including travel in countries other than Portugal. This was close to the cost of obtaining similar material in the United States.

At the close of the 1951 work all supplies were stored at Palma. A field survey was made over much of the infested area, and conditions appeared favorable for further collections the next year if necessary.

In 1952 preparations were made for another collection program in Portugal. I left the United States in early April and after a short period in France and Spain proceeded to Portugal, where I set up a project under almost the same conditions as in the previous year. The Portuguese Government again made available supervisory personnel and means of transportation. A larger space was provided in the building furnished by the owners of the Estate of Palma, and some improvements were made in the laboratory set-up as the result of experience from the first season's work. The entire personnel entered into the program the second year with enthusiasm, and highly satisfactory results were obtained.

The collecting area was much smaller because an intensive spraying campaign carried on by the Portuguese Government and large Estate owners had eliminated several of the smaller outlying infestations. Spraying in Portugal is done mainly with modern mist blowers. Heavy defoliation occurred again over much of the remaining area, and full advantage had to be taken of all good collection zones.

Results of the second season's work in Portugal were much better than those of the first year. From 564,000 pupae 350,000 female tips, or 62 per cent, were obtained. This is the highest percentage ever recorded in the years that collecting and rearing work has been carried on. The cost per tip was reduced to 2.7 cents, one of the lowest on record. Included in the over-all program costs was the expense of sending an assistant to Portugal from the United States for the later stages of the collecting and rearing work. The total number of employees used this second year was 34, an increase of 4 over the preceding year. The season for collecting was about the same each year, running through the month of June.

Two of the major problems of collection programs in this country are absent in Portugal—namely, the larval and pupal parasite *Sturmia scutellata* and the polyhedral virus disease commonly known as wilt. Although there are numerous parasites of the gypsy moth in Portugal, including many native ones not present in this country, *S. scutellata* is seldom recovered there. In the United States many pupae are collected in which these parasites are developing, with consequent loss of pupae in the rearing laboratory. In some seasons this loss is heavy. Only a small fraction of 1 per cent of the pupae collected in the last two years in Portugal have been lost through parasitism by *Sturmia*. The wilt disease has not appeared in any part of the infested area south of Lisbon during this time. According to C. M. Baeta Neves, of the Superior College of Agronomy in Lisbon, no outbreak of the wilt disease has ever been known to occur in this area. The absence of wilt is probably the main reason for a continuation of gypsy moth outbreaks of epidemic proportions year after year in the district south of Lisbon, which covers about 400,000 acres. The long, dry season starting about the first of May, with little or no rainfall during most of May and June, and the cool nights during this period probably have much to do do with absence of outbreaks of wilt.

According to Spanish forestry officials, two extensive gypsy moth outbreaks occurred in southern Spain during the past season. One area of about 20,000 acres is in the Province of Gibraltar, east of Cadiz. The other area is north of Barcelona along the shores of the Mediterranean. There is also a heavy infestation in the Forest of Mamora near Rabat in French Morocco. Very heavy outbreaks causing widespread defoliation have occurred in Yugoslavia in the last two years. Thus, it appears quite certain that collecting grounds may be found in Europe and North Africa in future years if necessary.

Isodrin, Endrin and Lindane for Grub Control in Turf

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The new insecticides isodrin[1] and endrin[1] are stereoisomers of aldrin and dieldrin respectively. Their empirical formulae $C_{12}H_8Cl_6$ and $C_{12}H_8Cl_6O$ respectively are identical to those of aldrin and dieldrin.

The investigation reported here concerns the use of isodrin and endrin in fairway turf experiments. They were used in dosage series in 1951 and in replicated dosage series in 1952 in comparison with lindane to determine their comparative values in controlling mixed *Popillia japonica* and *Anomala orientalis* grub populations. The latter species predominated in the area in which the tests were made. The grub population was much lower during the seasons of 1951 and 1952 than formerly (Schread 1948, 1949).

METHODS AND MATERIALS.—A suitable area of grub infested fairway turf was selected for the experiments in 1951, and rough adjacent to the same fairway in 1952. One-eighth acre plots were staked out for the 1951 and one-sixteenth acre for the 1952 tests. To designate the corners of the plots 18-inch wooden stakes having 1.5 inch square tops and taper-

[1] Names approved by The Interdepartmental Committee on Pest Control of the United States Department of Agriculture.

Table 1.—Grub control in turf using isodrin and endrin in August, 1951.

Actual Toxicant per Acre	No. Sq. Ft. Dug	Isodrin No. of Live Larvae: Date	1.	2.	Endrin No. of Live Larvae: Date	1.	2.
0.5 lbs.	2	Aug. 29*	10	17	Aug. 20*	8	13
	2	Oct. 2	4	16	Oct. 2	3	18
	2	Nov. 14	1	1	Nov. 14	0	1
1.0 lbs.	2	Aug. 29*	13	11	Aug. 20*	3	10
	2	Oct. 2	3	4	Oct. 2	0	15
	2	Nov. 14	2	0	Nov. 14	0	3
2.0 lbs.	2	Aug. 29*	7	11	Aug. 20*	7	9
	2	Oct. 2	0	12	Oct. 2	5	1
	2	Nov. 14	1	0	Nov. 14	0	0
Untreated	8	Nov. 14	49	28			
Untreated 1952	4	Sept. 25	32	4			

* Before treatment.

ing to a point were driven into the turf so that the tops were flush with the surface of the ground.

Grub populations were determined in advance of treatment by removing sections of turf 1 foot square and 6 inches deep. These were made at the rate of 24 per acre. Table 1 gives the August 20 and August 29, 1951 and table 2, April 25, 1952 records of grub diggings before treatments.

In the 1951 experiments one per cent isodrin and endrin dust formulations were used at the rate of 0.5, 1.0 and 2.0 pounds of actual toxicant per acre. The dusts were mixed with a 6–2–0 granulated organic fertilizer as a diluent at the rate of 250 pounds per acre to achieve more uniform distribution of the insecticides. A 3-foot fertilizer distributor calibrated to assure the required number of pounds per each eighth acre plot completed the equipment. The endrin was applied on August 20 and the isodrin on August 29. Parallel ribbons 3 feet wide of insecticide-fertilizer formulations were crisscrossed over the plots to make certain all ground area was coated with the toxicants. The August 20 treatments were made on a cloudy humid day. Air temperature averaged 72° F. The August 29 treatments were made on a clear day with average air temperature 75° F.

Isodrin and endrin were used in the 1952 experiments as 18.5 per cent and lindane as 12.9 per cent emulsifiable concentrate each at the rate of 0.5 and 2.0 pounds of technical insecticide per acre. The treatments were made during a rainstorm using a 300 gallon hydraulic sprayer at 400 pound pressure for the purpose. A single nozzle gun and 50 feet of high pressure hose completed the equipment. The emulsions were applied in water at the rate of 400 gallons per acre.

RESULTS.—The population of grubs before and after treatment is recorded in tables 1 and 2. Isodrin endrin and lindane gave good control of *Popillia japonica* and *Anomala orientalis* grubs in fairway turf. It will be observed that the spring treatments in 1952 killed grubs more rapidly than the fall treatments in 1951. For the most part the April treatments were applied to grub populations that had moved upward from lower winter hibernation depths into grass roots relatively close to the surface of the ground. On the other hand the August treatments were applied to turf infested with grubs that had remained close to the surface of the ground from the time of hatching. Most of the April population was made up of third instar grubs whereas those present at the time of the August treatments were first instar grubs with a few early seconds and no thirds.

By comparison, soil temperature and size of grubs appeared to be less important than rainfall in the more rapid reduction of grubs in treated turf in April and May and the slower results obtained in late summer. Soil temperature was higher and the grubs were smaller during August and September than in the spring.

From August 20 the date on which the 1951 experiments were started until the final checking of the treatments on November 14, a period of 12 weeks, rainfall amounted to 9.56 inches. Of this amount 1.26 inches fell between August 20 and October 1 and the balance 8.3 inches from October 1 to November 14, that is, subsequent to the time most of the grubs had commenced to work down in the soil for winter hibernation. In contrast, rainfall including and following April 25, on which the 1952 treatments were applied, to the time the treatments were checked for results on May 29, amounted to 7.15 inches. The spring treatments were made during a rainstorm totalling slightly over one-half inch.

The summer treatments of 1951 were first of all made with low concentrate dusts. Secondly, almost no precipitation occurred in August following their use and only 1.06 inches during September. Consequently only a minimum of insecticide penetration occurred before October. In contrast the April 1952 treatments were made with emulsions in 400 gallons of water per acre on a stormy day. In addition more than 6.5 inches of rain soaked the plots during the subsequent 5 weeks.

As a result there were a few living grubs in all but one plot 12 weeks after the August 1951 treatments with isodrin and endrin, but none 5 weeks subsequent to their use in April 1952. Untreated plots were well infested with both species of grubs at all times.

Plot examinations during the last week in September 1952 showed all of both the 1951 and 1952 isodrin, endrin and lindane treated areas to be free from grub infestation. In contrast, adjacent untreated turf in fairway and rough revealed mixed grub populations averaging 8.9 per square foot.

There was no noticeable difference in the rate of kill between the two species of grubs. Furthermore from the standpoint of ridding the turb of grubs during 5 to 12 weeks following treatment the amount

Table 2.—Grub control in turf using isodrin, endrin and lindane in spring, 1952

Actual Toxicant per Acre	No. Sq. Ft. Dug	Date	Isodrin No. of Live Larvae		Endrin No. of Live Larvae		Lindane No. of Live Larvae	
			1. *Popillia*	2. *Anomala*	1. *Popillia*	2. *Anomala*	1. *Popillia*	2. *Anomala*
0.5 lbs.	4	April 25*	22	28	18	20	31	33
	4	May 29	0	0	0	0	1	0
	4	Sept. 24	0	0	0	0	0	0
2.0 lbs.	4	April 25*	23	35	18	42	16	24
	4	May 29	0	0	0	0	2	1
	4	Sept. 24	0	0	0	0	0	0
Untreated	4	May 29	15	53				
Untreated	10	Sept. 24	37	52				

* Before treatment.

of actual isodrin and endrin in the range of 0.5 to 2.0 pounds per acre did not appear to be significant. In the first series of experiments in the spring of 1952 (but not in the replicated ones) there were a few living grubs on the lindane plots, but none following use of isodrin and endrin at comparable dosages (Table 2). This would appear, however, not to be an important difference.

SUMMARY.—The new insecticides isodrin and endrin and lindane, used as emulsions and in dust at dosages of from 0.5 to 2.0 of actual toxicant per acre, gave good control of *Popillia japonica* and *Anomala orientalis* grubs.

April treatments provided much faster control than August ones. The differential appeared to be due to heavy precipitation in the spring, with comparatively none in late summer and early fall. Hence rapid or slow penetration, as the case may be, of the insect toxicants to the area of the turf where the grubs were feeding. This resulted in accelerated kill of the populations on the one hand and delayed mortality on the other.

Lindane was about as efficient as isodrin and endrin in ridding turf of grubs in one month in midspring. In the fall of 1952 all treated plots were entirely free from grubs whereas untreated turf contained an average of 8.9 grubs per square foot.

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DDT for Earworm Control in Sweet Corn

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A brief review of the literature on control of corn earworm, *Heliothis armigera* (Hbn.), in sweet corn (Wilson *et al.* 1952, in Florida; Eden 1952, in Alabama; Rutschky 1952, in Pennsylvania; Ditman 1950, in Maryland; Kulash 1950, in North Carolina; Anderson *et al.* 1952, in California; and Blanchard *et al.* 1951) shows that recommendations on rates, methods, and number of applications vary considerably. The recommended number of applications varies from two to five. Rates of DDT vary from one to two pounds per acre per application both with and without mineral oil. Methods of application vary from the use of hand equipment to airplanes.

Three experiments were conducted in 1952 to procure information on some of these problems under Alabama conditions.

EXPERIMENT 1.—Experiment 1 included DDT sprays of two particle sizes and DDT dust applied by airplane, and DDT and DDT-mineral oil emulsion sprays applied with ground machinery. The plots consisted of 4-acre blocks in a field of Golden Cross Bantam Evergreen sweet corn. The treatments were unreplicated, but four samples were taken from each plot.

Stearman planes were used for the aerial applications. The dusting plane had an opening on each side of the fuselage. The spraying plane was equipped with rotary discs for breaking the spray into specific sizes; no nozzles were used. The ground sprays were applied with an Iron Age potato sprayer equipped with two regular potato spray nozzles per row. The spray was applied at 250 pounds pressure and 17.5 gallons per acre.

The plots receiving DDT by airplane received four pounds of DDT per acre per application both as dusts and sprays. Sprays were applied at 50 and 300 micron size at 4 gallons per acre. Forty pounds of 10 per cent DDT dust was applied to the plot receiving dust. The plots sprayed with ground equipment received two pounds of DDT per acre per application. One spray was a commercial 25 per cent DDT concentrate; the other was WE-5 Corn Earworm Spray,[1] which was a DDT-mineral oil formulation.

Applications were begun on May 26, at which time 10 per cent of the stalks were in silk. The weather was very hot and dry, and the corn silked unevenly. To provide sufficient coverage of new silks, five applications were made at 3-day intervals. Applications were made on May 26 and 29 and on June 1, 4, and 7. The corn was harvested on June 12. One hundred ears were examined at four places in each plot.

Results of Experiment 1 are presented in table 1. All treatments resulted in significantly more wormfree ears than no treatment. Although there was no difference between the 50 and 300 micron size aerial sprays, both sprays were significantly better than the dust applied by plane. Both sprays applied by ground machinery were much more effective than those applied by plane even though only one-half as much DDT was applied. The DDT emulsion was as effective in control of the earworm as the DDT-mineral oil emulsion. During the latter part of the experiment there were extremely high temperatures and low humidity. Slight foliage burn occurred on the corn receiving the DDT-mineral oil sprays. No burn was evident on the corn sprayed with DDT alone.

EXPERIMENT 2.—Experiment 2 was designed to determine when to start spraying and the number of applications at 3-day intervals that would be required for practical earworm control. It consisted of four spray treatments and an untreated check on Golden Cross Bantam Evergreen corn. Spraying was begun just before silking and continued for 4 and 5 applications on two plots; it was begun at 10 per cent silking and continued for 4 and 5 applications on two other plots. Plots consisted of 4 rows across a 40-

[1] Thompson-Hayward WE-5 Corn Earworm Spray containing 0.8 pound of DDT and 4.32 pounds of mineral oil per gallon.

Table 1.—Comparison of aerial and ground application of DDT sprays and dusts for control of corn earworm in sweet corn. Bon Secour, 1952.

TREATMENT[1]	WORM-FREE EARS[2]	
	Per Cent	Angle
None	5.5	13.32
Aerial spray, 300 microns	30.3	33.04
Aerial spray, 50 microns	27.3	31.24
Aerial dust	14.8	22.52
Ground spray, DDT alone	73.3	58.94
Ground spray, DDT-mineral oil	73.8	69.27
LSD, 5% level	—	6.76

[1] Plots treated by airplane received 4 pounds of DDT per acre per application; those treated with ground equipment received 2 pounds of DDT per acre per application.
[2] Mean of 4 samples.

acre field. Treatments were replicated four times. The corn was sprayed with a commercial[1] corn earworm formulation at the rate of 2 pounds of DDT and 10.8 pounds (approximately 1.5 gallons) of mineral oil per acre per application. The sprays were applied with a high-clearance self-propelled sprayer equipped with 4 fan-type nozzles[2] per row. Sprays were applied at 125 pounds pressure and at the rate of 20 gallons of spray per acre. The first sprays were applied on June 16. The corn was harvested July 2.

Results of the experiment are presented in table 2. All spray treatments resulted in over 90 per cent worm-free ears as compared to only 22 per cent on the unsprayed corn. However, there were no differences between the spray treatments. Under the conditions of this experiment four applications were sufficient. Whether spraying was begun before silks began to show or after 10 per cent were out was not reflected in the controls obtained. There were no effects from the sprays on how well the ears were filled out. There was slight foliage burn on the plots receiving five applications of spray.

EXPERIMENT 3.—Experiment 3 was designed to compare the effects on earworm control of varying amounts of DDT and mineral oil in emulsion sprays. DDT was tested at 1, 2, and 3 pounds per acre per application with 1.5 gallons of mineral oil. The rate of mineral oil was tested at 0, 1.5, and 2.5 gallons per acre per application with 2 pounds of DDT.

Four sprays were applied at 3-day intervals beginning on June 18 when 10 per cent of the stalks were in silk; the same equipment was used as in Experiment 2. The source of DDT was a commercial 25 per cent emulsifiable concentrate.[3] The corn was harvested on July 2.

Results of the experiment are given in table 3. All of the spray treatments resulted in significantly more worm-free ears than no spray. The check had only 18 per cent worm-free ears whereas the lowest for sprayed corn was 72.5 per cent. When the mineral oil rate was constant at 1.5 gallons per acre, the two pounds of DDT was significantly better than one, but three pounds was not significantly better than two. When the rate of DDT was constant at 2 pounds per acre, varying the amount of mineral oil did not significantly change the control obtained. Two pounds of DDT alone resulted in as good control as when 1.5 or 2.5 gallons of mineral oil was added.

There was no effect from the sprays on how well the ears were filled out. No foliage burn was evident where the DDT alone was used. Injury was slight on plots sprayed with 1.5 gallons of mineral oil and severe where 2.5 gallons was used.

Table 2.—Effect of different times and number of DDT-mineral oil sprays on control of corn earworm in sweet corn. Corte Farm, 1952.

TREATMENT	WORM-FREE EARS	
	Per Cent	Angle
None	22.0	27.93
4 Applications, beginning presilk	93.0	74.68
5 Applications, beginning presilk	92.8	74.68
4 Applications, beginning 10% silk	91.5	73.34
5 Applications, beginning 10% silk	90.0	71.94
LSD, 5% level	—	4.36

Table 3.—The effect of varying amounts of DDT and mineral oil in sprays for control of corn earworm in sweet corn. Corte Farm, 1952.

TREATMENT	FOLIAGE BURN	WORM-FREE EARS	
		Per Cent	Angle
None	None	18.0	25.05
1 Lb. DDT; 1.5 gal. oil	Slight	72.5	58.48
2 Lb. DDT; 1.5 gal. oil	Slight	85.8	67.90
3 Lb. DDT; 1.5 gal. oil	Slight	90.0	72.16
2 Lb. DDT; no oil	None	81.5	64.69
2 Lb. DDT; 2.5 gal. oil	Severe	87.3	69.26
LSD, 5% level	—	—	6.01

SUMMARY.—Results of three experiments on control of corn earworm in sweet corn in Alabama are presented. Aerial applications of DDT dusts and sprays were not as effective as sprays with ground equipment even though twice as much insecticide was used. There was no difference between the controls obtained with aerial sprays of DDT of 50 and 300 micron particle size. Aerial sprays were more effective than aerial dusts. In sprays applied with ground equipment there were no differences in the controls obtained between 4 and 5 applications whether spraying was begun before silks began to show or after 10 per cent of the stalks were in silk. Two pounds of DDT per acre per application was significantly better than one pound when applied with 1.5 gallons of mineral oil per acre. The control obtained with 3 pounds of DDT was not significantly better than that obtained with 2 pounds. The use of DDT alone resulted in as good earworm control as when used with 1.5 or 2.5 gallons of mineral oil per acre. None of the sprays used influenced how well the ears were filled out. There was no foliage burn when DDT was used alone, but injury did result when mineral oil was added.

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[1] Thompson-Hayward WE-5 Corn Earworm Spray containing 0.8 pound of DDT and 4.32 pounds of mineral oil per gallon.
[2] Tee-jet 650067.
[3] Shell Horticultural Oil No. 7 with Atlox Emulsifier.

Insecticides for Killing Cherry Fruit Fly Maggots Within the Fruit[1]

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A post-harvest spray is a highly recommended part of a successful cherry fruit fly, *Rhagoletis cingulata* Loew, control program in eastern Washington. Maggot infestations frequently approach 100 per cent of the unpicked fruits. These late infestations are due to a combination of factors, including inferior control practices during the harvesting period, late emerging flies, and flies dispersing from nearby untreated or partially treated trees or orchards.

A lead arsenate spray has long been the standard post-harvest treatment. For the last two years, however, several growers and commercial insecticide applicators have repeatedly reported maggot kill within cherries sprayed with parathion. Sherman (1951) sprayed pin cherries and sour cherries with parathion and found that one pound of 15 per cent parathion per 100 gallons of water gave excellent kill and that two pounds per 100 gallons gave complete kill of maggots in the fruit. Cox (1952) found that 1.5 pounds of 15 per cent parathion did not give complete maggot kill while one pound of 25 per cent parathion did. *EPN-300*, at 1 pound of the 27 per cent wettable powder per 100 gallons, also gave complete maggot kill. These reports confirmed growers' statements.

Neither Cox nor Sherman mentioned the presence or absence of breathing holes made by the maggots through the cherry skin. From 1 to 5 days before emerging from the cherry, the maggots open one or more holes about 0.06 inch in diameter. The majority of the holes are formed 3 days before emergence. The holes increase maggot mortality in two ways: by fumigation, and by the feeding of the maggot upon the sprayed skin.

1949 EXPERIMENT.—Two trees, one Stark's Gold and the other English Morello, were sprayed with 4 pounds of BHC (6 per cent gamma) per 100 gallons on July 5, at which time the fruits were heavily infested with maggots. Samples of 400 cherries, half from each variety, were collected and the fruit split open to determine maggot mortality (Table 1). No maggots were killed in cherries that lacked breathing holes through the skin. BHC is of limited value as a control for the maggots in the fruit at economic rates of application.

1951 EXPERIMENT.—This was a small-scale laboratory test designed to compare three insecticides as to the ability of each to kill maggots in Bing cherries containing breathing holes. Thirty cherries were used per treatment. These were divided into three lots of 10 each. Materials and rates per 100 gallons were as follows: 10 pounds (10 per cent gamma) BHC, 1 pound 25 per cent parathion, and 2 pounds 25 per cent malathon. The 10 cherries were then sprayed with 5 cc. of the insecticide mixture. Each cherry was thoroughly wet.

Table 1.—Mortality of maggots in 400-cherry samples from trees sprayed with 4 lbs. BHC (6% gamma) per 100 gal. Prosser, Wash. 1949.

DATE	MAGGOTS ALIVE	MAGGOTS DEAD	MAGGOTS KILLED
			%
July 5 (pre-spray)	275	0	0
July 6	252	43	14
July 7	288	25	8
July 8	338	43	11

Table 2.—Mortality of maggots in 30-cherry samples sprayed with various materials in a laboratory test, Prosser, Wash. 1951.

	MATERIAL AND CONCENTRATION PER 100 GALLONS			
	BHC, 10 Lb. 10% Gamma	Malathon, 2 Lb. 25% W.P.	Parathion, 1 Lb. 25% W.P.	Check Clear Water
Maggots alive	7	7	0	10
Maggots dead	7	2	10	0
Maggots pupated	2	1	5	9
Maggots killed, %	42	20	67	0

After being sprayed, the fruits were hung by the stems above a thin layer of soil so that none were in contact. After 48 hours the fruits were split and the soil sifted (Table 2). Not every cherry contained a maggot, but the data indicate that parathion was relatively effective in killing maggots in cherries that have breathing holes through the skin.

1952 EXPERIMENT, PROSSER.—Three Bing cherry trees were chosen that had 24 fruit-laden branches within 8 feet of the ground. These branches were divided into 8 replicates of three adjacent branches each and the three treatments assigned at random to the branches. By July 15 many of the fruits had breathing holes but very few of the maggots had yet emerged.

The sprays were applied on July 16 with a compressed air sprayer of three gallons capacity. Parathion, at the rate of 1 pound of 25 per cent wettable powder per 100 gallons and *Systox* at 1 pint of 32.1 per cent emulsifiable liquid per 100 were used. All

[1] Scientific Paper No. 1177, Washington Agricultural Experiment Stations, Pullman. Project No. 791.

[2] The help rendered by R. E. Bry and D. E. Johnson, Research Aids, is gratefully acknowledged.

Table 3.—Mortality of maggots in fruits 1 and 5 days after spraying. Prosser, Wash. 1952.

	MATERIALS AND CONCENTRATION PER 100 GALLONS		
	Systox, 1 Pt. 32.1% Emuls.	Parathion, 1 Lb. 25% W. P.	Check, Unsprayed
One day after spraying			
Total Fruits	50	64	68
Total maggots	50	56	64
Maggots dead	23	18	0
Maggots killed, %	46	23	0
Five days after spraying			
Fruits with breathing holes			
Total fruits	30	97	101
Total maggots	18	43	74
Maggots dead	11	30	4
Maggots killed, %	61	70	5
Fruits without breathing holes			
Total fruits	273	430	299
Total maggots	51	46	63
Maggots dead	44	37	5
Maggots, killed %	86	81	8

Table 4.—Numbers of maggots pupating 1 and 5 days after spraying. Prosser, Wash. 1952.

	Systox, 1 Pt. 32.1% Emuls.	Parathion, 1 Lb. 25% W. P.	Check, Unsprayed
One day after spraying			
Total fruits	50	64	68
Pupae	12	8	61
Pupae per 100 fruits	24	12	90
Five days after spraying			
Total fruits	303	527	400
Pupae	6	7	147
Pupae per 100 fruits	2	1	37

fruits were thoroughly wet with the sprays.

Twenty-four hours later samples of fruit, each with a breathing hole, were taken from each treatment. Not more than 20 fruits were picked from any one replicate. Several replicates in each treatment were short of such fruit, and all cherries that could be found were taken. The fruits in half of each sample were split open and all maggots checked to determine mortality (Table 3). The fruit in the other halves of the samples were placed, by treatment, on quarter-inch mesh hardware cloth above a thin layer of soil. Each cherry was placed so that it did not touch any other cherry. On August 2, the soil was sifted and pupal counts were made (Table 4). Both the maggot and the pupal counts show that a substantial mortality was obtained in 24 hours after spraying.

On July 21, five days after the spray application, as many fruits as possible were collected. Again, half were split open immediately and half placed upon hardware cloth. The fruits to be split were divided into those cherries with breathing holes and those without (Table 3). The samples placed on hardware were not divided according to the presence or absence of breathing holes (Table 4). Mortalities obtained after a 5-day lapse of time were superior to those of the 1-day period. Maggots without breathing holes suffered as high a mortality as did those with breathing holes.

1952 Experiment, Wenatchee.—The efficacy of parathion as a post-harvest spray was evaluated under field conditions. Unharvested Lambert cherries, heavily infested with maggots, were sprayed with 1 pound parathion 25 per cent wettable powder per 100 gallons of water on August 1. The dilute concentration of spray material was applied by means of a speed sprayer with a blower attachment at 300 pounds pressure. Four treated trees in an area not subject to drift were selected for sampling of cherries. One week after application of the spray material, samples of cherries were picked at random and immediately examined for living maggots. Only those maggots which were assumed to have died recently, based upon a pearly white appearance, were recorded. All others, assumed to have died from natural causes such as asphyxiation and lethal temperatures developing in shriveling fruits, were excluded from consideration.

The data presented in table 5 show high mortalities of maggots in fruits resulting from the application of parathion. The highest mortality obtained in treated fruits was 98 per cent and the lowest was 64 per cent. In both of these groups the trees were sprayed on one side only. However, in the latter, wind interfered with the application, while in the former, the tree was located in a site protected from air turbulence. Field examination for dead maggots indicated the presence of both second and third instar larvae in approximately equal numbers and one very small maggot, probably a first instar. The dead maggots were found throughout the cherries. As large numbers of cherries without visible breathing holes contained dead maggots as of those with breathing holes.

Table 5.—Mortality of maggots in fruits of treated and untreated Lambert cherry trees, Wenatchee.

Tree	Parathion, 25 Per Cent Wettable per 100 Gals. Water	Mortality per 50 Maggots Examined	
		Number	Percentage
1	1	48	96
2	1	49	98
3	1	32	64
4	1	47	94
5	untreated	3	6
6	untreated	1	2

This test indicated that relatively effective economic control of cherry fruit fly maggots in cherries was achieved with one pound of 25 per cent wettable parathion per 100 gallons of water applied with a speed sprayer. More efficient control, especially in those portions of trees not adequately covered by a speed sprayer, probably could be achieved by means of a hand spray gun.

Discussion and Conclusions.—BHC and malathon resulted in substantially lower mortalities than either parathion or *Systox*. BHC apparently kills by fumigation and contact, since maggots in fruits without breathing holes were unharmed.

The data show that parathion and *Systox*, at the strengths used, gave about equal mortality of maggots. The 1-day mortality was considerably lower than that obtained 5 days later. Both materials caused high mortality of maggots in fruits without breathing holes, suggesting that both materials may be absorbed into the fruits. It is possible that the insecticides penetrated through the skin and then acted as fumigants, causing maggot mortality by toxic vapors in the tunnels in the flesh. Maggot mortality, expressed by pupal counts, confirms that the 1-day mortality is substantially inferior to that obtained after 5 days.

The similarities between the life cycle of the black cherry fruit fly, *Rhagoletis fausta* O.S., and the species involved in these studies suggest that the two species might be controlled equally well in the fruits in the maggot stage by use of parathion.

The 1952 field evaluation of parathion at 1 pound of the 25 per cent wettable powder per 100 gallons applied with a speed sprayer indicated that this material can be very effectively used as a post-harvest spray.

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Forest Insect Survey of Maine

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The forest insect detection work in Maine was inaugurated by the State Entomologist in 1921. Maine was probably the first state to start such a survey. The actual field work is under the direction of the State Entomologist whereas the identification, handling and recording of collections is handled by the author.

The purpose of the Forest Insect Survey of Maine is to show what insects are present on each species of tree, their relative numbers from year to year, the stages and time each is present, and so far as practical their relationship to the trees and to one another. Most of the material is being collected by some 190 men whose primary work is fire detection and suppression but who have as a required part of their work assistance with insect and disease control work. This fire control organization is set up to cover completely all of Maine. Each warden is supposed to make a minimum of 11 collections each season distributed in as many townships as possible in his district. Collections are also made by six regular Forest Insect Rangers, the entomological staff, and by cooperators. The rangers spend much of their time on special collections, projects, plots and other work. Employment of regular rangers provides experienced personnel to lay out and check permanent plots, to secure data on species fluctuations in definite areas and to carry on other special investigations. They are also available for help on control projects. Because of the threat of outbreaks by the European spruce sawfly and more recently by the spruce budworm, emphasis has been placed on collections from spruce and fir. Regular collections are made by spreading a 6 by 9 foot beating sheet under a favorable tree and beating with a 10-foot pole as much of the tree on it as possible without changing the position of the sheet. Then all insects are put in a mailing tube, omitting ants and spiders. For each collection two or three trees of one kind are beaten and the insects put in one tube. A field report blank is filled out for each collection giving the collector, town, definite locality, date, tree species and number beaten, and other information and this report blank is sent with the insects.

On receipt at the laboratory the collections are separated into ranger, warden and other groups and arranged by collector and number. The field report is checked especially for locality and host and dated the day of receipt. Then all insects are picked out of each collection and divided on the basis of taxonomic group, or stage, in preparation for identification. Initial identifications are all written on the back of the field report concerning each shipment. Identification work, compilation and filing of records, and summaries on species or conditions are all facilitated by consistent methods of recording determinations and other data. The taxonomic groups to which the insects belong are roughly followed. Determinations are regularly listed starting with the sawflies and other Hymenoptera, then the Lepidoptera, Coleoptera and other groups. Material is determined to species as far as possible at the time it is received, and much of that which is not specifically determined is mounted or reared in order to secure determination at a later date. Rearing work necessitates checking the recorded host and in the case of many miscellaneous larvae suggesting the proper food or possible foods for testing. Rearing work is commonly necessary to obtain the parasites, to secure detailed biological information, to complete partial identifications and to secure adult specimens of many species.

Another type of collection is secured with light traps. These are distributed over central and northern Maine to try to find out if flights of spruce budworm from the heavily infested areas in Canada come into Maine. Part are placed on mountains or in isolated places and have used either kerosene or Coleman lanterns to produce light. The others use electricity. Most of these traps are essentially a large funnel opening into a metal can or large glass jar, with the light over the funnel. A 2 inch layer of plaster of paris or cellulose sponges is wet with sufficient carbon tetrachloride or other killing agent to kill the insects caught. A few box-like traps of the improved Rothamstead pattern have been used recently and make bigger catches than the other types. These use a similar arrangement for kiling the catch. During the most favorable nights so many moths come to the best traps that the moths need to be removed once during the night. The trap operators are supplied with reinforced cigar boxes, which are filled with alternating layers of cellucotton and newspaper. The moths caught during one night are spread out thinly enough to prevent molding on one or more layers of the cellucotton. A locality and date label is placed with each layer and a sheet of newspaper on top of each layer of moths. Boxes are mailed as soon as they are full. On receipt at the laboratory boxes of moths are checked for mold, pests, etc. These collections are examined for all the more important forest insects and a record made of each. This gives accurate records of the time when a species flies in different parts of Maine and a working knowledge of the relative abundance of each species. Developing infestations are often detected by means of the light trap material before they are discovered by the field collectors. This year of advance information may be important in making a check on a developing infestation. The light traps also give information on the spread of a pest from known centers of infestation.

Determination of the material beaten or collected from the trees is the most difficult problem of the survey. During the three busiest months in 1951 an average of 35 mailing tubes of insects was received per day. These tubes contained an average of 4.92 species and 12.39 individuals. In other words, 172.1 specific determinations of 432.92 individuals were made per day, or nearly one specimen per minute. The determination work is all done by one individual which limits its completeness. All specimens which are recognized or can be checked to species are determined to species. Unknown larvae of sawflies and Lepidoptera are commonly reared in an attempt to complete determinations. Other species are determined to genus, family or order. An attempt is made to secure determinations on all tree-feeding insects, their parasites, predators and commonly associated forms; therefore much material is submitted to specialists. All of these records are compiled for permanent files following a systematic arrangement. Determination of the light trap material requires considerable time. From 21 traps in 1952 about 70 cigar boxes of insects were received. Recognizing the important tree-feeders in this bulk of material is time-consuming. Much helpful information on both field and laboratory methods has been received from workers on the Forest Insect Survey of Canada. Reference collections of the adults and other stages,

and a library are necessary both for making determinations and for other phases of the work. Larvae are inflated or preserved in alcohol and adults are mounted. These are identified and arranged as far as possible to facilitate comparison and identification work.

This forest insect survey has developed working methods for collecting, rearing, determining and recording forest insects, their parasites and predators. It will supply important information on the presence and absence of pest species, and on the species and their numbers to be found on the different kinds of trees from year to year. Biological data, records of distribution, and specimens for taxonomic and other work are also secured.

Topical Application of Insecticide Solutions to *Anopheles quadrimaculatus*

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The standard technique for obtaining comparative toxicological data of chlorinated hydrocarbon insecticides against adult mosquitoes has been by exposure to treated surfaces. Valuable information of practical importance such as suitability of formulation, rate of application, rapidity of knockdown, repellency, and effects of climatic conditions and of the physical and chemical properties of the surface upon the insecticidal residue can best be obtained from this method of testing. Fay *et al.* (1949) have demonstrated, however, that periods of contact with residual deposits of DDT are quite irregular and of short duration in the case of *Anopheles quadrimaculatus* Say. Likewise, Ludvik *et al.* (1951) found after a short period of forced exposure that many *A. quadrimaculatus* would leave a DDT-treated room when possible. Under these conditions, significant survival of sufficient duration (72 hours) to permit oviposition occurred. In addition, numerous, continously confined mosquitoes were able to seek out as resting places such untreated surfaces as curtains and furniture after having been in contact with the DDT deposit for only a short time. This contact was often of such short duration that lethal doses were not picked up. When closely confined to a treated surface by means of a petri dish section, however, 100 per cent mortality would occur within 15 to 30 minutes. Thus, it is apparent that the period of exposure, the degree of confinement, and the amount of untreated surface available, as well as the evenness of the residual deposit, all affect the results obtained by panel testing.

It was therefore believed that if a more precise method of application could be developed, or an existing technique adapted for use with mosquitoes, data could be obtained which would be more suitable for drawing comparisons both between various insecticides against one species and between several species against one insecticide. In addition, it would then be possible to chemically analyze a group of mosquitoes treated with a known amount of DDT, for instance, to determine whether or not it was being metabolized to other compounds.

Various techniques have been developed by a number of workers for carrying out the topical application of insecticide solutions to such insects as houseflies, roaches, milkweed bugs, grasshoppers, etc. Most of these techniques utilize a machine to produce small volumes of liquid similar to that described by Trevan (1922). Carbon dioxide anesthesia to immobilize the insects to be treated is often an important step in these techniques. March & Metcalf (1949), for instance, have described a method for treating houseflies in which they applied one microliter of acetone solution of DDT to immobilized flies.

Since adult mosquitoes are relatively small, fragile, and very active, immobilization is mandatory. By trial and error, it was found that caged *Anophales quadrimaculatus* and *Aëdes canadensis* could tolerate an exposure of 30 seconds to carbon dioxide gas which flowed into the "knock-out" chamber (a 6 by 8 inch battery jar) at the rate of 0.3 to 0.5 cubic feet per minute. Lower or higher flow rates would not give satisfactory immobilization, while longer exposure periods proved to be fatal to the insects.

It is necessary to pick up the mosquitoes by the wings with forceps, the legs being much too fragile to permit such treatment. Female *Anopheles quadrimaculatus* are much easier to handle than are males since the apices of the wings extend slightly beyond the apex abdominis. In males, on the other hand, the apices of the wings and the apex abdominis are essentially contiguous. With practice and care, however, males can be handled quite rapidly without causing injury to the abdomen. This operation can best be done with both sexes while the anesthetized individuals lie on one or the other of their pleural surfaces. In this situation, the wings are usually folded as in the normal resting position over the dorsum of the abdomen. Due to the short period of anesthetization, it is possible to treat a group of only about 15 individuals at a time.

One cubic millimeter of acetone, benzene, cyclohexanone, 1,4-dioxane, and ethyl alcohol (95 per cent) was found to be highly toxic when applied to the dorsum in both sexes of *Anopheles quadrimaculatus*. When the volume was reduced to approximately 0.5 cubic millimeters, alcohol produced combined anesthetic-solvent ("check") mortalities of 5 and 15 per cent in females and males, respectively. Acetone was too volatile to use at this low volume and the other solvents again produced high mortalities. A subsequent reduction of the volume of alcohol to about 0.25 cubic millimeters greatly reduced the check mortality.

Figure 1 shows the machine now being used in this laboratory for making topical applications of insecticides to mosquitoes. The liquid reservoir is a 0.25 cc. tuberculin syringe equipped with a 27-gauge hypodermic needle. The needle shaft is bent down 90° and the point ground flat. In this machine, the drive wheel rotates to impel the plunger screw, but both the wheel and driving lever are stationary with respect to planar displacement. The drive wheel was made from a watt-hour meter serrated disk in which is centered a brass bushing. The drive wheel and bushing are mounted to the base through the brass disk which is located on the right side of the wheel as shown in the figure. The apparatus was calibrated by setting the lever stops and weighing the amount and calculating the volume of mercury discharged for a definite number of strokes of the driving lever. The keyed sliding block was placed between the screw and syringe plunger to eliminate lateral play of the screw upon the plunger. The knurled knob at the left end of the sliding block locks the screw and prevents its rotation as the drive wheel turns. When the

[1] Acknowledgment is made of the assistance given by R. E. Sparkman who designed and made the solution dispensing machine used in this project. The author is also indebted to the Rohm & Haas Co., E. I. du Pont de Nemours & Co., Julius Hyman & Co., and the American Cyanamid Co. for supplying certain insecticides used in this investigation.

syringe is empty, this knob is released and the screw retracted manually.

This machine was designed for left hand operation to permit manipulation of the insects with the right hand. In addition, the lever and stop arrangement allows full attention to be centered on the insects and eliminates the necessity for matching micrometer markings carefully in order to rapidly obtain repeated, predetermined volumes of solution.

In preparation for testing, the sexes were separated in the pupal stage on the basis of differences in the genitalia (Keener & Hess[2] 1943; Moorefield 1951), and the number required were placed in about one-fourth inch of water in the bottom of an emergence cage. Figure 2 shows how pint ice cream cartons were utilized as emergence cages. Two days after the pupae had been placed in the cages, the bottoms were replaced so that the adults would not drown. This operation removed the pupal exuviae from the cage and also facilitated subsequent handling. The adults were fed by placing squares of cotton soaked with sugar water on the screen at the top of the cage. Tests were carried out 5 days after the pupae had been placed in the cages. The adults were considered to be four days old when treated even though the actual ages ranged from three to five days. Mortality counts were made 24 hours after treatment. Mosquitoes were considered to be alive if they were able to fly and could cling to the wall of the cage even though they might still possess only one or two legs. Forty-five to 60 individuals were used for each dosage level.

This procedure and equipment were used to obtain dosage-mortality data which were recorded on logarithmic probability paper. Curves were fitted by eye to the points plotted and the LD_{50} and LD_{90} values read from the graph. These data are presented in Table 1.

Table 1.—Toxicity[1] of several insecticides to four-day old adult *Anopheles quadrimaculatus* by topical application in 0.5 cubic millimeter of ethanol.

Insecticide	LD_{50}[2]		LD_{90}[2]	
	Male	Female	Male	Female
p,p'-DDT	0.020	0.066	0.045	0.13
p,p'-TDE	0.041	0.10	0.098	0.22
Methoxychlor (tech.)	0.035	0.10	0.078	0.22
Chlordane[3]	0.105	0.24	0.19	0.46
Dieldrin	0.009	0.023	0.022	0.048
Lindane[3]	0.0085	0.011	0.032	0.042
Toxaphene[3]	0.15	0.29	0.29	0.5
Malathon	0.0087	0.0095	0.019	0.022
Allethrin	0.0029	0.008	0.013	0.041

[1] Data corrected for check mortality by means of Abbott's formula.
[2] Micrograms of insecticide per individual.
[3] A.A.E.E. reference standard.

The slopes of the dosage-mortality curves of DDT, TDE, methoxychlor, chlordane, dieldrin, toxaphene, and malathon are essentially parallel as are those of lindane and allethrin. The slopes of the curves for lindane and allethrin are much steeper than the others, however. When the toxicity of DDT is assigned a value of unity, as shown in table 2, it is readily apparent that dieldrin, lindane, allethrin, and malathon are considerably more toxic than DDT to *Anopheles quadrimaculatus*. Also, there is little difference shown between the relative

Table 2.—Relative effectiveness against *Anopheles quadrimaculatus* of several insecticides as compared to DDT.

Insecticide	LD_{50}		LD_{90}	
	Male	Female	Male	Female
Toxaphene	0.13	0.23	0.16	0.26
Chlordane	0.19	0.28	0.24	0.28
p,p'-TDE	0.49	0.66	0.46	0.59
Methoxychlor (tech.)	0.57	0.66	0.58	0.59
p,p'-DDT	1.0	1.0	1.0	1.0
Dieldrin	2.2	2.9	2.0	2.7
Lindane	2.4	6.0	1.4	3.1
Allethrin	6.9	8.3	3.5	3.2
Malathon	2.3	7.0	2.4	5.9

LD_{50} and LD_{90} values for one sex or between the two sexes for either dosage level. However, exceptions to these generalizations are to be noted for lindane and allethrin, the LD_{90} levels being "less effective" than the LD_{50} levels. There is also a marked difference in the toxicity of lindane and malathon to male and female *A. quadrimaculatus*. The toxicity of *p,p'*-methoxychlor would be expected to more nearly approach that of *p,p'*-DDT than does the technical product used in these tests.

SUMMARY.—Adult mosquitoes can be utilized as test insects for the topical application of insecticide solutions if a group of 15 individuals are anesthetized for 30 seconds when the flow rate of the gas is adjusted to 0.3 to 0.5 cubic feet per minute. The insects must be handled for treatment by grasping the wings with forceps. Ethanol is a satisfactory insecticide solvent for use with mosquitoes as long as the volume used does not exceed 0.5 dubic millimeters. A machine for measuring small liquid volumes which is manipulated with the left hand and which is provided with lever stops in order to deliver a predetermined volume will contribute much toward making this procedure successful. LD_{50} and LD_{90} data for *Anopheles quadrimaculatus* have been obtained by topical application for toxaphene, chlordane, TDE, methoxychlor, DDT, dieldrin, lindane, allethrin, and malathon.

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Some Acarological Techniques

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Mites are such small animals that special methods are often needed to handle them. The following techniques may prove helpful to acarologists and students of other small arthropods.

Fixatives often cause the flexion of legs beneath the body of mites and small insects, which increases the difficulty of microscopic examination of these forms and some of their parts. Even hot water frequently causes this flexion. If chemical reagents are used, however, one must be careful to prevent excessive shrinkage or swelling of the specimens.

Since many mites possess a coating on the exoskeleton which causes them to float in hot water, the writer uses a killing agent which permits the immersion of the mites, offsets the flexion of the legs, and decreases the time involved in dehydration and clearing following fixation. This is the K.A.A.D. mixture of Peterson (1943), intended for use with immature insects. The solution effects fixation of oribatid, tyroglyphid, tetranychid and hydrachnid mites with good results. The color is lost in some of the pigmented forms, however.

The above solution effects the fixation of the mites within five minutes, although specimens may remain in it for several hours without deleterious effects. The solution kills quickly. The body parts contract, then swell slightly and finally relax. The legs remain in an extended lateral position, which facilitates examination of subsequent microscopic mounts. The legs of orbatid mites sometimes become slightly, but the general effects of the reagent are good.

The use of this fluid has another distinct advantage. The specimens are washed in several changes of 95 per cent alcohol to remove the fixative. They may then be stored in 70 per cent or 80 per cent alcohol; they may be transferred to clearing agents, such as beechwood creosote or methyl salicylate for balsam mounts; they may be mounted directly in Berlese fluid. In each instance there is a distinct advantage in the time involved in preparation. Plate shrinkage of phthiracarid mites is lessened by taking the specimens to 35 per cent alcohol before mounting in Berlese fluid. This modification may also be required in a few other instances. In such cases experimentation will indicate changes in technique.

The writer also uses a dry-mount technique for mounting oribatid mites to demonstrate bristles. In some instances this is successful. Usually, however, the specimen becomes too opaque for the clarity of bristle insertions and some external structures unless such mounts are viewed by direct light. Transmitted light is insufficient in most instances.

The latter technique consists of the fixation and dehydration used for balsam mounts. The excess clearing fluid is blotted from the specimen and the latter is then placed in a dry cell on a slide. The cell is constructed by cementing small pieces of coverslip glass to the slide in the shape of a square. Small-bore glass or celluloid tubing may also be cut into lengths and used as cells. The specimen is attached to the slide by means of a minute drop of balsam—just enough to hold the mite in the desired position. After orientation of the specimen, balsam is placed around the cell and a coverslip is added. The balsam seals the coverglass in place and a bubble remains in the cell around the mite.

Small brushes are ideal for handling mites. The writer uses medical applicators, fine hair, thin-walled glass tubing and household cement to construct small brushes with which to handle mites. The glass tubing is tapered in a flame and cut into one-half inch lengths of which the larger end is about one-eighth of an inch in diameter. The tips of the medical applicators are trimmed to fit into these tapered glass sleeves. Several small hairs are inserted in each sleeve and held in place with the fingers. The tapered end of the applicator is dipped in cement and carefully inserted into the glass sleeve as the hairs are held. After the cement dries, the brush is trimmed to the desired size. The opposite ends of the applicators are marked with different colors and certain brushes are used in certain reagents. The writer uses different brushes for each of the tasks of picking up mites from soil samples or other material, transferring them to alcohol, transferring them to clearing solutions. The clearing solutions damage the brushes most rapidly, which necessitates frequent replacement.

When Berlese funnels are used, mites are caught in dry jars attached beneath the funnels. The contents of these jars are emptied intermittently into watch glasses. The tip of a small hair brush is moistened with water and touched to the individual mites as they are observed under a dissecting microscope. The mites are then transferred to vials, fixed, dehydrated and mounted. This pick-up technique is especially good for oribatid mites, as they are kept relatively free of soil particles. It also facilitates the separation of individual species into separate vials, which is more difficult when water or alcohol is used to catch the mites below the funnels.

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Sap Beetles in Maryland[1]

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Within recent years sap beetles, particularly *Carpophilus lugubris* Murr. have caused concern among sweet corn growers in Maryland. On occasion they have infested all of the ears in a sweet corn field. Actual damage to the kernels was seldom great. Just before, and at the time of harvest, adult beetles were frequently encountered in numbers in corn ears beneath the husks. Developing larvae were frequently found in the same areas as the adults. Many of the larvae were found feeding deeply between the rows of the kernels, and a few actually within the kernels. Such conditions of infestation reduces the value of sweet corn when it is placed on the market and places an extra burden on canners in culling operations before processing.

Because of the increasing importance of this insect as a pest of sweet corn in Maryland, studies were begun in 1949 in an attempt to find an effective means for its control. Early surveys led to the opinion that there existed a distinct relationship between heavy sap beetle infestations and damage from certain other insects, particularly the Japanese

[1] Scientific Article No. A387. Contribution No. 2403 of the Maryland Agricultural Experiment Station (Department of Entomology).

beetle, the European corn borer and the corn earworm. These surveys also indicated that DDT properly applied seemed to be giving a considerable measure of sap beetle control in fields sprayed to control the Japanese beetle. Heavy infestations of sap beetles were practically always associated with other insect damage, while corn treated with DDT for Japanese beetle control seldom suffered the same intensity of infestation. On the other hand a corn field was occasionally encountered which gave contradictory evidence. Corn ears not injured by other insects were infested by sap beetles, and DDT sprayed corn was heavily infested.

In an attempt to evaluate these contradictory observations, work was undertaken to determine the relationship between sap beetle abundance and damage from other insects. This work revealed that adult sap beetles frequently entered corn ears that showed no evidence of being attacked by other insects. The number of sap beetles found in uninjured ears were usually small, but nevertheless they were present. Ears in all stages of development from early silk to maturity were found that harbored sap beetle infestations. A test in which undamaged corn ears were covered with cheesebloth, to prevent other injury, showed definitely that sap beetles may enter undamaged ears and cause damage. In a field of sweet corn heavily infested with Japanese beetles, 175 undamaged ears were carefully selected and covered with cheesecloth. The silks in this field were dead at the time of covering. The covers were removed over an 8-day period. Twenty per cent of these ears were infested by sap beetles in the egg, larval, or adult stage. This finding is similar to the results reported by Knowlton (1948). He reported that 40 per cent of the ears in a field of sweet corn were infested by sap beetles, of which 25 per cent of the ears showed no previous damage by other insects.

In spite of the above findings there is much evidence that tends to indicate sap beetles prefer corn that has been attacked by other insects, or injured in some way. Heavy sap beetle infestations always seemed to be prompted by injury from other insects. Ears damaged by other insects frequently had as many as 50 sap beetle larvae present, while ears not previously damaged rarely showed an infestation of more than four or five larvae. In many of the spray tests where corn was sprayed with DDT to protect it against Japanese beetle damage, the sap beetle infestations were negligible, while in the unsprayed, or check plots, the sap beetle infestations were severe.

As already noted, observations on fields sprayed with DDT for the control of other insects indicated that this insecticide may have been effective also for sap beetle control. Subsequent tests using DDT directly for sap beetle control and in fields where Japanese beetles were not a problem, gave very erratic and inconsistent results. These tests indicated that DDT was not killing the sap beetles that came into contact with it. It was not uncommon to find adult sap beetles active in freshly sprayed ears. Following these results, laboratory tests were undertaken to observe the efficiency of DDT for killing sap beetles when placed in direct contact with it. These tests indicated that this insecticide was not efficient for killing the beetles, at least when exposed to it continuously on impregnated disks.

In making the tests adult sap beetles were collected one day prior to being used. The beetles were held overnight at a temperature of 45° F. which inactivated them sufficiently for handling, and did not increase normal mortality. The insects were then exposed on circular cardboard disks which had been impregnated by dipping in DDT solutions of various concentrations. The insects were confined to the disks in petri dishes continuously for a period of 24 hours. Ten beetles were used in each test and each test was replicated 10 times. These tests indicated that DDT was a very poor insecticide for killing adult sap beetles. On disks dipped in a DDT solution containing as much as 3 pounds of actual DDT in 100 gallons of water the mortality of the beetles over a 24-hour period averaged only 25 per cent.

Following the disappointing results obtained with DDT, similar tests were made using several other chlorinated hydrocarbon insecticides as well as several phosphorus base insecticides. The chlorinated hydrocarbon insecticides included: aldrin, dieldrin, methoxychlor, TDE, and toxaphene. The phosphorus base insecticides included: EPN (Ethyl-p-nitrophenyl thionobenzene phosphonate), NPD (Tetra-n-pyrophyl dithienopyrophosphate), and metacide.

With methoxychlor at a dosage of 2 pounds in 100 gallons of water, no kill at all was obtained. At the same dosage level, the kill from TDE, averaged 28 per cent. Aldrin at a dosage of 0.5 pound killed 97 per cent, while dieldrin at the same dosage level killed 66 per cent of the beetles.

Lindane and toxaphene showed some promise as insecticides for killing sap beetles. Lindane at a dosage of 0.25 pound killed an average of 93 per cent of the insects, while toxaphene used at a dosage of 2 pounds killed 90 per cent. In the tests with the phosphorus base insecticides, EPN at the rate of 1 pound of the active ingredient in 100 gallons of water killed 80 per cent of the beetles while NPD at the same rate killed 100 per cent of the insects. In every instance when metacide was used 100 per cent mortality was obtained. This material was used at the rate of 0.4 of a pound in 100 gallons of water. Three tests were made on as many days, all replicated 4 times, but in each test all the controls died. It is believed that in setting up the experiments, the checks in some way came into contact with metacide or were possibly exposed to the fumes of this insecticide, which escaped into the laboratory. Where the other materials were used the survival of the checks was good.

SUMMARY.—Evidence was obtained which shows that on occasions sap beetles do infest corn not injured by other insects, but from the work done it is not possible to give a clear-cut answer to the extent to which this may occur. Generally speaking, heavy sap beetle infestations were associated with other insect damage. On the basis of observations in fields treated for other insects with DDT there seemed to be some value in the treatments for the control of sap beetles. However, laboratory tests indicated that DDT is of little value against sap beetles. Of the other materials tested in the laboratory, aldrin, toxaphene, lindane, EPN and metacide were effective and should be field tested.

Influence of Parathion Wettable Powder on Effectivness of DDT

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Influence of various spray combinations containing DDT has been the subject of investigation at this station for a number of years. In the course of these investigations, a small amount of parathion wettable powder has been used with DDT each season, beginning in 1948. The purpose of the investigations has been to measure the initial and residual toxicity of the spray deposit to codling moth larvae.

DDT under orchard conditions gradually looses effectiveness against codling moth and other insects (Cox 1951; Gunther *et al.* 1946; Hough 1945). Under similar conditions effectiveness of parathion is lost rapidly, usually within a few days,[1,2] King *et al.* 1949; Mitchell *et al.* 1948). Experience has indicated that in seasons favorable to the development of codling moth adequate control by DDT may not extend longer than two weeks. Consequently, the need to extend the period of effectiveness of this compound is important wherever the insect persists as a major apple pest.

METHODS.—Conventional power spraying equipment was used to spray apple trees of the York Imperial variety. About 25 gallons of diluted spray mixture was applied to each tree in each application. Most of the trees averaged 12 to 14 feet in height. DDT, parathion and the indicated combination of these materials were used in each application beginning not later than the second cover spray. Tests on duration of effectiveness followed the fourth (late June) and the fifth (late July) covers. To test initial toxicity apples were removed from the trees in each experimental plot one day after spraying and subjected to the attack of codling moth larvae at the insectary. Removal of the spray deposit by handling was largely avoided by using scissors to cut the apple stem from its point of attachment to the fruit spur. A small wire sieve held immediately below the fruit caught each apple as it was severed from its attachment. The apples were brought to the insectary in galvanized buckets from which they were removed by grasping the stem with a display hook. The display hooks were then used to suspend the fruit from racks designed for this purpose. In this manner 40 apples showing conspicuous spray deposit were collected from each experimental plot for each test. Similar samples for the tests on residual toxicity were collected at weekly intervals up to 4 weeks after spraying. The first series of tests followed the fourth cover spray in late June and the second series, from the same trees, followed the fifth cover spray applied during the last week in July or not later than August 1. Ten days after the eggs had hatched, each fruit was carefully examined to determine the number of injuries (stings and live entries) and then cut open to determine the number of live larvae. At the same time the number of hatched eggs was determined for each fruit, the average being 9 to 11 per apple. All of the DDT wettable powder used was manufactured by E. I. du Pont Company and all of the wettable parathion powder by the American Cyanamid Company. Wettable powder formulations containing 50 per cent DDT and 25 per cent parathion were used prior to 1952, but in 1952 we used 75 per cent DDT powder and 15 per cent parathion powder.

RESULTS.—The results summarized in Tables 1 and 2 show that a small amount of wettable parathion powder added to DDT increased initial toxicity and residual effectiveness of the spray deposit as compared to DDT alone. Data reported by Newton & List (1949) indicated improvement of control by 50 per cent DDT powder plus a small amount of parathion as compared to DDT alone.

Increased initial toxicity of DDT plus parathion might be explained by the insecticidal action of parathion, but all of the improvement in effectiveness demonstrated two weeks and later following each spray is difficult to attribute to insecticidal action of

Table 1.—Duration of effectiveness of DDT and parathion applied in late June of 1948 to 1952 inclusive, showing the average per cent of damage to apples picked at the indicated interval after spraying and then exposed to attack of codling moth larvae.

Interval After Spraying		Amount of Active Inredients in 100 Gallons and Average Per Cent of Codling Moth Damage		
		DDT 12 oz.	DDT 12 oz. and Parathion 1 oz.	Parathion 6¼ oz.
1 day	Live larvae	1.19	0.19	0.27
	Total injuries	2.66	2.02	7.13
1 week	Live larvae	2.95	0.44	1.73
	Total injuries	7.15	4.15	16.49
2 weeks	Live larvae	9.34	3.75	6.39
	Total injuries	14.04	9.56	27.35
3 weeks	Live larvae	10.48	6.52	10.88
	Total injuries	14.23	13.51	24.12
4 weeks	Live larvae	17.56	12.56	13.82
	Total injuries	22.30	19.74	32.04

Increased initial toxicity of DDT plus parathion might be explained by the insecticidal action of parathion, but all of the improvement in effectiveness demonstrated two weeks and later following each spray is difficult to attribute to insecticidal action of

[1] Anonymous. 1950. Parathion data presented to U. S. Food and Drug Administration. Brief on behalf of American Cyanamid Co. Pp. 10–14.

[2] Anonymous. 1951. Parathion insecticides. Grower's handbook. Pennsylvania Salt Mfg. Co. Pp. 21.

Table 2.—Duration of effectiveness of DDT and parathion applied in late July of 1948 to 1952 inclusive, showing the average per cent of damage to apples picked at the indicated interval after spraying and then exposed to attack of codling moth larvae.

Interval After Spraying		Amount of Active Ingredients in 100 Gallons and Average Per Cent of Codling Moth Damage		
		DDT 12 oz.	DDT 12 oz. and Parathion 1 oz.	Parathion, 6¼ oz
1 day	Live larvae	0.44	0.13	0.00
	Total injuries	1.78	1.13	2.48
1 week	Live larvae	0.88	0.36	0.32
	Total injuries	1.72	1.71	5.20
2 weeks	Live larvae	3.12	1.35	2.11
	Total injuries	6.06	5.03	12.40
3 weeks	Live larvae	6.04	2.61	3.81
	Total injuries	12.19	7.23	21.26
4 weeks	Live larvae	4.54	3.94	6.66
	Total injuries	7.87	7.58	27.38

parathion residue. Synergism is suggested, but another possibility may be that the physical state of the DDT mixture was altered in such a manner as to result in improvement of residual toxicity. In this connection it should be mentioned that the 75 per cent DDT formulation used in 1952 reacted differently in the DDT-parathion combination when tested three and four weeks after spraying than was obsesved in previous years when the 50 per cent DDT formulation was employed. The DDT-parathion combination of 1952 not only failed to give better control than DDT alone when tested three and four weeks after spraying, but on the contrary, live larvae exceeded the number found in apples sprayed with DDT alone (5.3 per cent in the 3 weeks' test and 5.4 per cent in the 4 weeks' test for the DDT-parathion combination compared to 0.6 per cent and 0.9 per cent respectively for DDT alone). In the tables presented, the observed difference in performance of the 75 per cent DDT powder of 1952 is obscured by averages established by 50 per cent DDT during the previous seasons. During the first two weeks of the 1952 tests DDT-parathion was more effective than DDT alone. Inert ingredients employed in formulating 75 per cent DDT wettable powder differs from the inert materials used in making 50 per cent wettable powder.

More effective control is indicated by the data shown in table 2 than in table 1. At least two factors are believed to contribute to the improvement demonstrated for the second series of tests. In the first place the average temperature was slightly lower throughout the month of August as compared to July. In the second place some spray residues from the previous cover sprays probably persisted.

SUMMARY.—Codling moth control tests demonstrated that initial and residual toxicity of 50 per cent DDT wettable powder was increased by adding a small quantity of wettable parathion powder to the spray mixture. Improvement in residual toxicity to the larvae was noted 3 and 4 weeks after the fruit was sprayed. Improvement of toxicity of 75 per cent DDT was not noted in 1952 more than two weeks following the spray application.

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Development of Resistance to Rotenone by the Mexican Bean Beetle

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The Mexican bean beetle has been controlled in Connecticut by use of rotenone dusts or sprays since the introduction of these materials almost twenty years ago. Minnum *et al.* (1952) have reported that vegetable growers have had some difficulty in controlling the insect with this insecticide. It seems possible that the Mexican bean beetle is developing resistance to rotenone.

Stocks of the Mexican bean beetle that had not been exposed to rotenone were not available for making comparative tests. However, tests of the comparative toxicity of rotenone and methoxychlor were made in 1945 (Turner 1946). At that time there was no reason to believe that bean beetles were resistant to rotenone. Methoxychlor is not yet in common use for control of the bean beetle in Connecticut. It was therefore postulated that bean beetles should not have developed a specific resistance to methoxychlor, and that another comparative test might furnish some information as to whether or not a strain resistant to rotenone has been developed. Unless there proved to be a cross-resistance for methoxychlor, the toxicity of methoxychlor would be increased relative to rotenone.

Bountiful beans were planted May 13, 1952. Individual plots were 10 feet of single row, and the treatments were randomized in blocks, with three replicates. Four concentrations of each insecticide were applied by means of hand dusters on June 18 and 24, during the hatching period of first-generation eggs. Results were obtained July 1 by counting the larvae surviving on each of 10 plants taken at random from each plot. The results were as follows:

MATERIAL	CONCENTRATION, PER CENT	NO. LARVAE	PER CENT REDUCTION
Rotenone (in cube root)	.125	103	66
	.25	84	73
	.5	31	90
	1.0	7	97
Methoxychlor	.625	53	83
	1.25	43	86
	2.5	33	90
	5.0	5	98
None	—	307	—

The results were plotted on the logarithmic-probability grid and curves fit by inspection (Fig. 1). Unfortunately the results following application of methoxychlor were somewhat variable. It is, however, possible to make an estimate of relative toxicity. At the 95 per cent level of control, from 2.5 per cent to 3.5 per cent methoxychlor is required to equal .8 per cent rotenone. The curve for rotenone is obviously steeper than the curve for methoxychlor, therefore, the relationship between the two materials changes with each level of control. In 1945, at the 98 per cent level of control, 1.0 per cent methoxychlor was as effective as .5 per cent rotenone (Turner

1946). In 1945, the two curves intersected at about .4 per cent concentration of each material. In 1952, they intersected at about 0.2 per cent concentration.

In other words, there was a greater difference in toxicity of these two insecticides in 1952 than in 1945. If the toxicity of rotenone had decreased to any marked extent because of the development of resistance to it, the difference in the two might be decreased rather than increased. Unfortunately nothing is known of possible cross-resistance to rotenone and methoxychlor.

Further evidence on the toxicity of rotenone is available by comparison of tests over a period of years. Turner (lit. cited) has published results of dosage tests of rotenone dusts on bean beetles, using the same diluent throughout the series. The general plan of these tests was identical. The first application of dust was made as soon as the larvae hatched from the eggs, and the second a week later. Mortality was

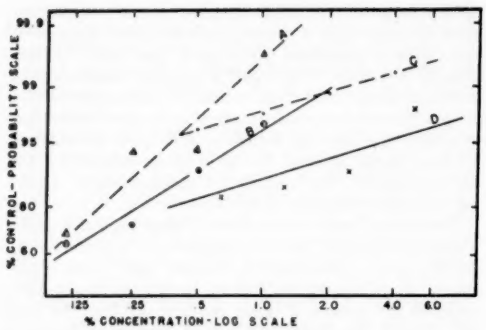

FIG. 1.—Dosage-mortality curves for field control of the Mexican bean beetle. A rotenone and C methoxychlor (Turner 1946); B rotenone and D methoxychlor in 1952.

determined by comparing the population on treated plants with that of untreated plants. Results of these in terms of dosage required to kill 90 per cent of the bean beetles were as follows:

Year	*Generation*	*L.D. 90*
1942	I	.09% rotenone
1942	II	.12
1943	II	.35
1945	II	.35
1945	II	.27
1952	II	.5

It is obvious that there has been a general trend upwards in the dosage required to kill 90 per cent of the larvae. The largest change came between 1942 and 1943, and represented a three-fold increase in dosage. The 1952 results represent a four-fold increase over 1942, and at most a two-fold increase over 1943. Unfortunately detailed data on the population of bean beetles in the years 1942 to 1945 is no longer available. Some of the changes in amount required to kill 90 per cent of the insects may be caused by changes in the population being treated. It must be stated, however, that the 1952 infestation was not severe, and was in fact much smaller than in some of the earlier tests.

The difference in dosage required is substantial but not necessarily alarming. It is of about the same magnitude as the increase in resistance of houseflies to pyrethrum developed by Bruce & Decker (1950) after exposure for 21 generations. Similar exposure to DDT produced flies requiring a dosage of about 800 times that necessary for normal flies for equal mortality.

The work of Beard (1952) implies that insecticides which lose effectiveness rapidly may not be able to select highly resistant strains from a population of insects. Rotenone loses its effectiveness within a week or 10 days in the field in Connecticut. Furthermore, rotenone has never been toxic enough virtually to eliminate bean beetles from a field. It is therefore less likely to select strains or mutants practically immune to it, as may be the case with the more effective insecticides.

The degree of resistance to rotenone in bean beetles (and to pyrethrum in houseflies) may be a sort of "survival of the most fit" rather than the result of selection of a resistant mutant. Whether or not this resistance will increase is of course unknown, but it seems probable that it will not reach the same degree as in the case of DDT on houseflies. The increase in dosage of rotenone required to kill the bean beetle has been substantial enough to justify consideration of other materials to control this pest.

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State Estimates as a Basis for Determining National Requirements for Pesticides

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The advanced technical knowledge of the use of pesticides, gained from research aimed primarily at the development of practical control measures, has made it possible for farmers and the public to be well informed as to what materials to use, and as to

when and how to apply them. The kinds and quantities of pesticides used in the United States have undergone a virtual revolution during the short period since the end of World War II, and the annual cost of chemicals now used to control pests is estimated at over one-half billion dollars. Yet, little attention has been given in the past to the determination of quantitative needs or economic demands for these products. Information on the quantities of major pesticides currently used by agriculture has not been available from agricultural sources.

Statistical information on the quantities of insecticides and fungicides used in 1934–35 was published by Roark (*1935*). At that time the total quantity of technical compounds, excluding diluents and additives, estimated to have been used as insecticides and fungicides was somewhat in excess of 215 million pounds. The quantity of technical chemicals estimated to have been used as pesticides in 1944 had grown to about 513 million pounds.[1] However, beginning in 1945 a vast change occurred in the kinds and quantities of chemicals used as pesticides, and the quantity of technical chemicals estimated to have been used in 1951 exceeded 1 billion pounds.

Late in 1950, a staff office was established in the Office of Materials and Facilities of the Production and Marketing Administration to study the pesticide situation and to develop reliable statistical data on the quantities of pesticides used by domestic agriculture. Such information, maintained on a current basis, is essential for estimating requirements for pesticides in advance of the time of actual need.

Studies were made in 1951 and 1952 under the sponsorship of the PMA State Committees and Insular Area Offices to provide basic information from agricultural sources for determining national requirements for major pesticides. The studies were generally made, in the state and insular areas, by special technical advisory committees composed of representatives from the State Land Grant College, Experiment Station, and Extension Service; State Department of Agriculture; State Livestock Sanitary Association; other Bureaus and Branches of the U. S. Department of Agriculture; and other sources having to do with crops, livestock, and markets. Representatives of the pesticide industry were consulted in many states. The data obtained from these studies were based on farmers' plans and preferences as evaluated by persons in the several states familiar with the local uses of and needs for pesticides.

This paper summarizes the state reports of the quantities of major pesticides estimated to have been used during the crop years 1949–50 and 1950–51. The crop year, for purposes of this survey, is the period beginning October 1 of one calendar year and ending September 30 of the following year. Pesticides are chiefly the chemicals or chemical mixtures which are used as insecticides, fungicides, and herbicides. Certain other chemicals used as defoliants for cotton and other crops are included.

Table 1 shows the total quantity of each of certain major pesticides, based on the combined State reports, estimated to have been used in 1949–50 and 1950–51. The domestic disappearance, at the producers' level, of each pesticide during the corresponding periods is also shown in this table. Domestic disappearance is calculated from production and inventory data on each material, corrected for exports and imports, during the period. Domestic disappearance consists of quantities disposed of in the domestic market by the manufacturers of basic pesticidal chemicals for all purposes, including use by agriculture and by the Armed forces.

In general, the combined State and Insular area reports on the quantities of pesticides estimated to have been used were in reasonably close agreement

[1] Agricultural Statistics 1945, U. S. Dept. Agr.

Table 1.—Quantities of major pesticides estimated to have been used during each of the crop years 1949–50 and 1950–51, in thousands of pounds.

Pesticide	Quantity Estimated to Have Been Used Based on			
	1949–50 State Reports	Disappearance During 1949–50	1950–51 State Reports	Disappearance During 1950–51
Benzene hexachloride[1] (includes lindane)	12,136	8,600	10,058	9,600
Calcium arsenate	42,191	38,789	24,462	39,276
DDT	69,709	57,638[2]	63,707	72,688[2]
Aldrin, chlordane, dieldrin, heptachlor, and toxaphene	59,369	40,000	75,050	60,000
Lead arsenate	37,340	28,600	30,343	31,509
Parathion	3,072	2,551	3,880	4,670
Rotenone, 5 per cent	5,634	9,847[3]	4,959	7,027[3]
Tetraethyl pyrophosphate (100 per cent)	1,592	—	1,040	1,376
Sulfur, ground	392,601	538,592[4]	336,864	400,000[4]
Copper sulfate	100,716	108,480	93,260	100,000
Dithiocarbamates	15,584	—	13,667	—
2,4-D (acid)	14,810	17,600	21,811	23,494
2,4,5-T (acid)	—	—	2,767	2,822
Sodium chlorate	—	33,500[5]	—	40,300[5]

[1] Gamma basis.
[2] Revised from previously published data.
[3] Imports of rotenone bearing roots.
[4] Disappearance during the calendar years 1950 and 1951, respectively, for all domestic uses of ground sulfur.
[5] Supplies for herbicidal and defoliant use only.

with domestic disappearances during the respective periods. It should be understood, however, that the quantities given for domestic disappearance at the producers' level do not take into account considerable variations in stocks existing in the distributional system or channels of trade below the producers' level. Thus, when the quantity estimated to have been used, based on the State reports, exceeds domestic disappearance, it could indicate that part of this quantity was derived from stocks held in the distribution system from the previous year. When domestic disappearance exceeds the quantity estimated to have been used, based on State reports, an accumulation of stocks in the distributional system is indicated. Frequently, domestic disappearance figures, such as these, are referred to as consumption, but this may be erroneous in the light of the unknown quantity held in the distributional system. Thought should be given to the development of appropriate means for estimating stocks remaining in the channels of trade at the end of the season.

The state reports have been of inestimable value for calculating requirements for pesticides (Shepard *1952 a*) and regional distribution of estimated usage of pesticides (Shepard *1952 b*). Knowledge of the total quantities of pesticides used nationally and of the proportions used in each region should assist in the distribution of current production to meet the needs of each region.

The estimated requirement for each pesticide is a statement of the quantity needed to provide protection under conditions at least as severe as those encountered during the recent history of the particular pesticide. The studies of the quantities of pesticides estimated to have been used, made in the States and Insular areas, also served as the basis for the statement of preliminary estimated requirements for pesticides in 1953 (Shepard *1952 c* and *1952 d*). It should be understood, of course, that these preliminary estimates are subject to revision when data on domestic disappearance during 1951–52 become available.

Two annual studies in the States and Insular areas to develop basic data on estimates of quantities of pesticides used for agricultural production have been successfully completed, and the committees which assisted in developing these data can take justifiable pride in their work. Reports on these studies were invaluable at a time when demands for pesticides were greater than the supply. Now that demand during the last year (1951–52) of relatively low usage and plentiful supply has been sluggish, the State reports on quantities of pesticides estimated to have been used in 1951–52 would round out the study of a full cycle in relation to supply and demand, and be particularly useful in making predictions under similar circumstances in the future.

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Three New Insecticides for Housefly Control in Barns [1,2]

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The failure of chlorinated hydrocarbons to give control of houseflies in many parts of the world has led to an intensified search for new materials to be used against this species. Three new chemicals developed and tested in Switzerland were studied this past summer in cooperative tests by workers in the Entomology Department at Rutgers and Geigy Company, Inc.

The three compounds are as follows:

1. Pyrolan. This is the common name assigned by Wiesmann (1) to experimental compound G-22008 (1-phenyl-3 methyl-pyrazolyl-(5)-dimethyl carbamate).

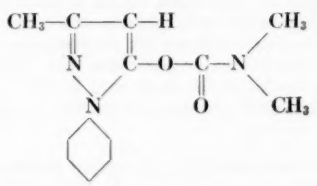
2. Pyramat. This is the common named assigned by Gysin (2) to experimental compound G-23330 (2-N-propyl-4-methyl-pyrimidyl-(6)-dimethyl carbamate).

3. Diazinon. This is the common name assigned by Gysin (2) to experimetal compound G-24480 (0-0-diethyl-0-(2-isopropyl-4-methyl-pyrimidyl (6)) thiophosphate).

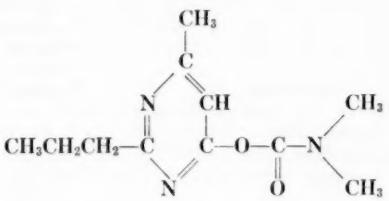
The structural formulas of the above compounds are shown in figure 1.

Pyrolan consists of colorless crystals which melt at 48–49° C. and is soluble at 0.2 per cent concentra-

Pyrolan



Pyramat



Diazinon

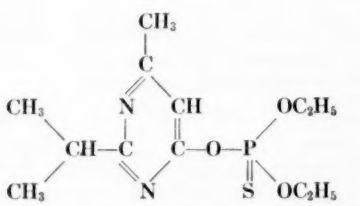


Fig. 1.—Structural formulas of the new insecticides.

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Department of Entomology.

[2] Paid publication requested by C. C. Alexander. Letter of Jan. 7, 1953.

tion in water at 20° C. and readily soluble in most organic solvents. Toxicological studies indicate that the oral LD-50 for this compound on laboratory rats and mice is 53.5 and 46 mg./kg. respectively. Pyrolan as well as the other two compounds possess strong anticholinesterase action.

Pyramat is a straw colored viscous liquid which is readily soluble in most organic solvents. The oral LD-50 for rats and mice is about 200 mg./kg.

Diazinon is a colorless liquid which boils at 83–84° C. It is soluble up to 0.004 per cent in water and readily soluble in most organic solvents. Preliminary toxicological investigations show that the oral LD-50 to rats is approximately 100 mg./kg., and to mice is between 50 and 100 mg./kg.

Since Wiesmann (1950) reported that Pyrolan gave long residual control of flies, when combined with a DDT formulation, it was decided to apply it with a similar insecticide, methoxychlor, in these tests. The two other compounds were applied both alone and with methoxychlor. All of the materials used in these tests were formulated as wettable powders except the Pyrolan in barn No. 3, which was formulated as an emulsion concentrate.

For tests of these materials representative barns were selected which had good but not excellent sanitation. The barns used were of various construction and size and were located in Monmouth and Middlesex Counties. Insecticides were applied as wettable powders, alone or in combination with methoxychlor to all walls and ceilings of the barn applying one gallon of spray to 400 square feet with a power sprayer operating at 100 to 150 pounds pressure. Care was taken to cover all feed, feed troughs, and watering cups before spraying.

Tests were made in two series—one series (Barns 1 to 5) where we had no detailed records of previous insecticide treatments and where housefly resistance to insecticides had not been noted and the second series (Barns 6 to 9) with a high degree of resistance in the fly population and detailed records of previous treatments for fly control.

Barn 1 is a small unpainted barn used for horses and a few beef animals at Rutgers. Barn 2 was the main piggery building also at Rutgers. Barn 3, located near Farmingdale, is a large unpainted barn used to house about 50 head of dairy animals. Barn 4 is a smaller unpainted structure also located in the vicinity of Farmingdale. Barn 5, located near Marlboro, consists of an unpainted wooden structure used for calves and a maternity barn with concrete walls and corrugated aluminum roof. Barns 6 to 9 are all located near Freehold. Barn 6 is constructed of cinder blocks and the ceiling is lined with sheet rock. The other three barns are wood construction and had been whitewashed previous to the start of the fly season in 1952.

Table 1.—Applications in barns where houseflies had no obvious insecticide resistance.

Barn No.	Treatment	Date	Days Fly Control
3	1% Pyrolan+1% methoxychlor	June 17	14
3	1% Pyrolan+1% methoxychlor	July 8	17
3	1% Pyrolan (emulsion)+methoxychlor	Sept. 2	16
2	2% Pyramat	June 17	21
4	1% Pyramat	June 17	2
2	1% Pyramat+1% methoxychlor	July 29	21
1	1% Diazinon	June 17	72
4	1% Diazinon	July 1	21
5	1% Diazinon+1% methoxychlor	July 20	24

Table 1 shows the treatments which were made with the three insecticides in the first group of barns and the fly control which resulted.

Pyrolan was not too effective for it gave control for only two weeks in combination with methoxychlor. When used alone at 2 per cent or in combination with methoxychlor at 1 per cent, Pyramat gave 3 weeks control but when used alone at 1 per cent concentration only 2 days of control resulted. In treating the barns it was noted that this material killed flies extremely rapidly and it is thought that this material has promise as a contact insecticide with quick knockdown, but even though three weeks of control resulted when Pyramat was applied as a residual spray, it was obvious after a day or two that flies were surviving and respraying would be necessary as soon as a build-up of adult flies took place. In two cases this took 3 weeks.

The third material, Diazinon gave excellent control for the entire season in the horse barn and gave 3 to 4 weeks fly control in dairy barns. In the horse barn large numbers of dead flies were found each week for the entire season and we found it difficult to believe that the Diazinon alone was giving these results. We were assured repeatedly, however, that no other materials had been used in the barn. In all barns where this insecticide was used some dying flies could still be found even when re-treatment was necessary.

The second series of tests were instituted in late July after housefly resistance had been encountered in a number of dairy barns where the same insecticide had been used for several years as reported by Hansens (1952). In these barns and in laboratory tests none of the chlorinated hydrocarbons tested gave housefly control. Following this failure other materials were tested including Pyrolan and Diazinon in combination with methoxychlor. Treatments and results of these tests are shown in table 2.

Table 2.—Applications in barns where houseflies were resistant to insecticides.

Barn No.	Treatment	Date	Days Fly Control
6	1% Pyrolan+1% methoxychlor	Aug. 12	3
7	1% Pyrolan+1% methoxychlor	Aug. 12	17
8	1% Pyrolan+1% methoxychlor	July 29	5
8	1% Pyrolan+1% methoxychlor	Aug. 12	6
9	1% Diazinon+1% methoxychlor	Aug. 15	30

It is evident that both materials gave kills of flies but Pyrolan did not last long enough for it to be considered a very promising material. On the other hand, Diazinon gave excellent results for a month at the height of the fly season and after this barn had received successively in 1952 two applications of lindane, one of methoxychlor, and one of lindane plus synergist without any fly control. Unfortunately Diazinon was available in quantities sufficient to treat only one of these barns having a resistant fly population but the material performed so well that it merits further testing with either resistant or non-resistant flies.

SUMMARY.—1. Diazinon was the best of the three experimental materials tested for the control of houseflies. The residual action extended for a maximum of ten weeks against non-resistant flies and four weeks against resistant flies.

2. Pyramat and Pyrolan do not have the necessary residual activity to be considered as economically feasible control of houseflies in dairy barns.

3. Pyramat may be used in combination with other insecticides utilizing the quick knockdown ability of this material.

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Stilbene and Comparable Materials for Dry-Wood Termite Control

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Out of the enormous number of chemicals commercially and experimentally available, one criterion for the selection for test of those most likely to be valuable in repelling termites is their identity with or their similarity to the constituents responsible for the natural resistance of some woods to termite attack. Thus, many quinones were tested (Wolcott 1947) because East Indian teak, *Tectona grandis* L., contained beta-methylanthaquinone, but without discovering any quinone anywhere nearly as repellent. Of the other wood constituents commercially

Table 1.—Days after submergence 10 minutes in solution before attack by the West Indian dry-wood termite, *Cryptotermes brevis* Walker.

DILUTIONS OF	0.002%	0.005%	0.01%	0.02%	0.05%	0.1%	0.2%	0.5%	1%	2%
Tectoquinone (beta-methylanthraquinone, from *Tectona grandis*)			3	6	24	36	45	536	uneaten 7 years	
Pinosylvin (3,5-dihydroxy-STILBENE, from *Pinus sylvestris*)			565	567	570	579	644	670	674	END
Dihydropinosylvin monomethyl ether			375	389	441	482				
Pinosylvin dimethyl ether			147	152	190	564	577			
Pinosylvin monomethyl ether			uneaten 2 years							
STILBENE ($C_6H_5CH{:}CHC_6H_5$)			330	481	528	537	823	826	843	END
Stilbene compounds:										
2,4,6-trinitro			62	76	148	213	471			
2,4-dichlor		39	149	268	275	277	uneaten over 2 years			
2,4,2′,4′-tetranitro		142	uneaten 10 months							
4,4′-dinitro		uneaten 10 months								
2,4-dinitro-2′,4′-dichloro		uneaten 10 months								
DIPHENYL							3	6	8	9
Diphenyline Oxide								3	11	12
Tetrachlordiphenyl					2	4	8			
Pentachlordiphenyl						7	8	12	16	
Hexachlordiphenyl							10	21	23	42
Hexachlordiphenyl trisulfide					5	6	7	8	10	
Nonachlorterphenyl					3	10	13	19	24	28
Diphenyl triketone					8	11	12	13	14	
Diphenyl Selenium					4	8	13	130	217	
AZOBENZENE ($C_6H_5N{:}NC_6H_5$)		98	135	142	uneaten 10 months					
BENZALANILINE ($C_6H_5CH{:}NC_6H_5$)		90	92	93	96	289	313	318	END	
Benzalaniline compounds:										
3,4-dimethoxy	12	18	29	32	34	39	46	52	END	
4-methoxy	36	47	58	294	316					
3-methoxy-4-hydroxy-5-chloro	196	200	253	268	270	272	273	278	END	
2,3,4-trihydroxy	7	8	12	15	18	22	27	53	END	
2-hydroxy	26	33	112	123	137	207	212	267	END	
4-hydroxy	284	285	286	287	289	294				
3,4-dihydroxy	102	114	128							
3,4-methylenedioxy	52	54	58	77	159	178				
3-methoxy-4-hydroxy	210	217	251	255	258	262	267	272	END	
2-nitro	52	161	166	168	178	268	278			
2-hydroxy-3-methoxy	224	299	300	301	308	314	335			
2,4-dihydroxy	237	uneaten 10 months								
4-chloro	300	304	325	346	403	406	444	447	END	
2,4-dichloro	uneaten 9 months									
Chrysin (5,7-dihydroxyflavone from *Pinus lambertiana*)			55	56	58	61	79	82	90	END
Pinocembrin (5,7-dihydroxyflavonone from *Pinus lambertiana*)			57	72	74	78	102	108	288	END
CHLOROPHORIN (from *Chlorophora excelsa*)			73	137	138	147	159	uneaten 10 months		
TAXIFOLIN (3,5,7,3′,4′-pentahydroxy-flavonone, from Douglas fir, *Pseudotsuga taxifolia*)			150	154	uneaten 2 years					
Ryanodine (from *Ryania speciosa*)			84	204	slightly eaten in 4 years					
Mahogany extractive P2		uneaten 1 year								
POMIFERIN (from *Maclura pomifera*)			323	330	334					
Iso-pomiferin dimethyl ether	72	108	154	155	156					
Pomiferin trimethyl ether	80	86	98	101	109	110	112	END		
OSAJIN	39	48	51	53	55	58	61	END		
Iso-Osajin trimethyl ether	47	51	59	63	68	73	END			
Tetrahydro-Osajin	96	165	180	182	187					

available, none appears to have appreciable value in preventing termite attack, and temporarily at least, the investigation based on this logical line of attack necessarily had to be discontinued.

Dr. Erik Erdtman, heading the Division of Organic Chemistry of the Royal Institute of Technology in Stockholm, Sweden, working on the most important product of that country—its coniferous woods, extracted from Scotch pine, *Pinus sylvestris* L., "two phenols, pinosylvin and its monomethyl ether, which are responsible not only for the non-digestibility of pine heartwood, but also for its high resistance to attack by wood-destroying fungi." (Lindstedt 1951). Local entomologists whom he had consulted regarding the possibility of testing these substances against the common Swedish wood-eating insects assured him that such an investigation would prove very difficult and most inconclusive, thus he was the more readily induced to furnish adequate amounts of these and other extractives for test against the West Indian dry-wood termite, *Cryptotermes brevis* Walker. Samples of flamboyán, *Delonix regia* (Bojer) Raf., a wood most susceptible to termite attack, when submerged for 10 minutes in a 0.01 per cent solution of pinosylvin, were initially toxic, and when no longer toxic, were found to be repellent to termite attack for 565 days, or a greater length of survival time than any other chemical previously tested at such great dilution. But subsequently the resistance of samples treated with greater concentrations was comparatively short-lived, and the sample impregnated with 1 per cent pinosylvin was eaten after 674 days. At the minimum concentration, however, pinosylvin monomethyl ether has not been attacked in 2 years, and it remains to be determined how much more permanently resistant to termites it will prove to be than is pinosylvin.

Chemically, pinosylvin is a 3,5-dihydroxy-stilbene, and stilbene itself has long been commercially available. Like pinosylvin, initially toxic to termites for several weeks, but later attacked at minimum dilution before pinosylvin, the sample impregnated at 0.5 per cent of stilbene was eventually eaten by termites more than 2 years after impregnation. Indeed, the value of stilbene as a termite repellent soon appeared so obvious that Dr. Erdtman had prepared in his laboratory various nitro and chlorinated compounds, most of which appear to be outstandingly effective, at least during the period of test during which they have been available.

Stilbene is a diphenyl-ethylene, the double ethylene linkage being of the very greatest importance in determining its value, as will be noted by comparison with the results obtained from tests with the directly linked diphenyl, and its numerous compounds, not one of which gave even the slightest promise (Wolcott 1947). Indeed, Dr. Erdtman thought the ethylene might be wholly or in part replaced with nitrogen, and therefore submitted azobenzene and benzalaniline, besides numerous derivatives of the latter, for tests with termites.

In the initial tests, a dilution of a minimum of 0.05 per cent was considered suitable for testing almost any chemical, and it indeed proved to be quite adequate as a starting point for the numerous compounds tested which had little or no termite-resistance. As fewer and fewer of such chemicals were tested, the minimum dilution adopted for the more resistant chemicals was one-hundredth of one per cent. But such a long period of time elapsed in the case of some of these chemicals before samples impregnated with even one-hundredth of one per cent were attacked that for the benzanalines compounds it was standardized at one five hundredth per cent. At such an excessive dilution one might expect the wood samples thus impregnated would be promptly eaten, and some few of them were, but others have not been attacked in 8 or 9 months, termites confined with such samples gradually dying of starvation than eat the impregnated wood. None of these benzanilines appears to be toxic but they certainly are repellent, and at such minute dilutions that the 5 per cent commercial application required for such a standard repellent as pentachlorphenol seems fantastically enormous.

None of the other characteristic constituents of woods obtained by Dr. Erdtman can begin to compare with some of his synthesized benzalanilines in preventing attack by termites, and indeed chrysin and pinocembrin are (as was expected and predicted by him) quite valueless. Chlorophorin, obtained from a tropical tree notably resistant to termite attack, is distinctly disappointing, but taxifolin, from Douglas fir (source of so much plywood that promptly becomes termite-infested in the tropics), proves to be surprisingly resistant. A sample of wood of the shrub *Ryania speciosa* Vahl. placed in test with other woods in a petri dish, not only repels termites but within a few days kills all of them, even though they have eaten none of the wood. Its extractive, commercially available as an insecticide, is correspondingly powerful. Apparently it does not attain sufficient size to be used locally in construction or as a cabinet wood in northern South America, where it is endemic. The chemists have not yet determined the chemical composition of ryanodine. Even less advanced are the investigations of Dr. Conrado Asenjo of the School of Medicine, University of Puerto Rico, on the extractive from West Indian mahogany which is responsible or its well-known resistance to termite attack.

As compared with the termite resistance of West Indian mahogany which has arbitrarily been placed at 80 (Wolcott 1950), that of Osage orange, *Maclura pomifera* (Raf.) Schneider, is 90. Prof. M. L. Wolfrom of Ohio State University, has been working for some time on the extractives of this wood and, at the suggestion of Dr. Erdtman, submitted some for test against termites. The crude materials he sent first proved to be of little value, but pomiferin was outstanding. Dr. DeLong had previously tested pomiferin and other pure extractives for toxicity, and found that they were not toxic to the insects used in his experiments, but the tests to date with dry-wood termites indicate that some at least are highly repellent to *Cryptotermes brevis*.

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Response of Insects to Black and White Light[1]

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Although extensive studies have been made to indicate the response of insects to lights of various intensities and qualities, including ultra violet, little information is available upon the reaction of insects to black or invisible ultra violet light with wave lengths between 3000 and 3800 Ångstroms.

To obtain such information two Minnesota light traps of similar construction were operated at State College, Pennsylvania, during 1951 and more extensively during 1952. The traps were hung 8 feet from the ground in similar locations. They were placed about 100 feet from each other and were somewhat shielded by intervening foliage. The location was not ideal for maximum catches as the position of the traps was determined by the power line and the convenience in handling the traps. They were operated by the author and great care was exercised to keep the killing jars clean and to obtain every insect that was caught. These traps were controlled by a switch that turned them on and off simultaneously. Operation varied from 8 or 9 p.m. to 4 or 5 a.m. depending upon the amount of daylight at various periods of the summer.

During 1951 low watt lamps were used. A two-watt General Electric S-14 neon glow ultraviolet lamp with a "black light filter" was compared with 7 and 15 watt white frosted filament lamps. During 1952 high voltage lamps were used. A 100-watt G.E. mercury vapor lamp, known as BH4, with a built in red-purple filter, was compared with 50 and 100 watt G.E. frosted filament lamps. A G.E. 89G140 transformer was used as ballast with the BH4 lamp.

Table 1.—Spectral energy distribution of the high wattage lamps used during 1952.[1]

Spectrum	Wave Length Angstroms	100 Watt Black Lamp	100 Watt White Lamp
Invisible ultra violet	3,000– 3,800	.831	.06
Visible violet	3,800– 5,000	.03	.90
Green & yellow	5,000– 6,000	.00	2.25
Orange & red	6,000– 7,600	.04	6.80
Infra red	7,600–50,000	.00	80.00
Total		.901	90.01

[1] Per cent of lamp wattage.

The BH4 lamp emits considerable invisible ultra violet light between 3000 and 3800 Ångstroms but very little light visible to man. The white frosted G.E. lamp, on the other hand, emits very little invisible ultra violet but considerable rays from orange to red and infra red. The qualities of these lamps are enumerated below. For simplicity they are referred to in the test as black and white lamps.

As far as insects are concerned lights of various sorts cannot be compared. The following figures represent only their responses to black and white lights. Although the wattage of these were at times the same, the intensities, rated in foot candles, were quite different. At equal distances and equal wattages the white light was ten times as intense as the black light. It is reasonable to assume that different results might be obtained by varying the wattage and thus the intensity. During 1952 a 100 watt black light was used throughout the summer while the white light was used at 50 and 100 watts.

To simplify the records the catches are arranged by families or larger groups. It is obvious that comparisons cannot be made entirely on this basis for

Table 2.—Catches of insects at low wattage lamps; 1951.

Insect	3 Nights July 8 to 10		4 Nights July 11 to 15	
	2 Watt Black	15 Watt White	2 Watt Black	7 Watt White
Tipulidae	15	31	8	18
Misc. Diptera	15	17	6	15
Chrysopidae	0	0	1	13
Hemiptera	0	51	0	23
Cicadellidae	7	126	5	158
Misc. Lepidoptera	95	89	20	33
Micro. Lepidoptera	0	0	23	56
Coleoptera	5	12	5	10
Hymenoptera	0	0	1	3
Misc. insects	330	649	229	1,796
Total	647	975	298	2,125

[1] Authorized for publication on Marh 17, 1953, as paper No. 1795 in the journal series of the Pennsylvania Agricultural Experiment Station.

Table 3.—Catches of insects at high watt lamps, 1952.

Group	32 Nights July 10–Aug. 20		20 Nights Aug. 21–Sept. 20	
	100 Watt Black	100 Watt White	100 Watt Black	50 Watt White
Culicidae	16	22	16	12
Tipulidae	41	19	17	23
Anthomyidae	67	54	8	6
Nematocera	2,678	2,691	1,168	870
Misc. Diptera	324	270	189	74
Neuroptera	19	31	1	19
Psocidae	158	193	16	7
Trichoptera	204	76	152	33
Hemiptera	271	1,643	18	111
Cicadellidae	4,648	9,300	133	396
Fulgoridae	37	3	24	18
Aphididae	202	85	10	16
Tortricidae	313	234	34	31
Noctuidae	189	181	131	236
Geometridae	45	42	2	5
Crambidae	797	791	229	252
Arctiidae	83	45	0	0
Sphingidae	8	4	0	0
Micro. Lepidoptera	870	666	122	80
Misc. Lepidoptera	283	217	12	10
Scolytidae	31	24	0	0
Lampyridae	15	43	0	0
Carabidae	159	233	20	18
Elateridae	83	63	1	0
Curculionidae	25	56	22	22
Staphylinidae	151	82	5	0
Scarabaeidae	47	78	3	2
Misc. Coleoptera	1,222	1,048	34	47
Hymenoptera	448	877	33	29
Total	13,434	19,071	2,400	2,317

Table 4.—Some moths taken during 1952.

Species	32 Nights June 10–Aug. 20		20 Nights Aug. 21–Sept. 20	
	100 Watt Black	100 Watt White	100 Watt Black	50 Watt White
Feltia subgothica	4	1	43	59
Cirphis unipuncta	45	33	4	7
Agrotis ypsilon	50	2	1	4
Carpocapsa pomonella	61	25	8	3
Nomphila noctuella	26	44	22	53
Halisidota tesellata	83	45	0	0

individual species are attracted at different periods of the summer and may respond differently to various lights. Unless otherwise stated, the comparisons are based on insects attracted to lights of equal wattages.

The Nematocera responded about equally to black and white lights. This group included the Chironomidae, Cecidomyidae, Mycetophilidae Anisopidae, and Psychodidae. No Tabanidae or Simuliidae were raken. Only a few Culcicoides were trapped and these were difficult to separate because of the quantity of material gathered. Among the miscellaneous Diptera, the Drosophilidae and Muscidae were most abundant. Other Diptera were taken in small numbers.

The Neuroptera included only Chrysopidae and Hemerobiidae.

In general the Hemiptera responded more freely to the white light. The Miridae constituted the most outstanding catches and *Trigonostylus ruficornis* was attracted in considerable numbers. From July 19 to August 1, 30 specimens were taken at black light and 832 specimens at white light. On June 23 a small black species *Plagiognathus politus* was common. Twenty-four specimens were taken at black light and 148 specimens at white light.

The Cicadellidae likewise responded more freely to white light. Numerous species were taken but *Empoasca fabae* predominated throughout July. Late in July and during August a larger species *Phlepsius nebulosa* was relatively more abundant.

Various species of Tortricidae were taken including *Carpocapsa pomonella, Arygrotaenia velutinana, Archips fervidana* and *Laspeyresia prunivora.* In general they responded about equally to black and white light. Although the catches of the codling moth were relatively small, they seemed to respond most freely to the black light.

Numerous species of Noctuidae were taken. All were counted but only those that came in comparatively large numbers were identified. On the whole the species came about equally to black and white light. Individual species, however, showed some differences. *Feltia subgothica* was strongly attracted to white light while *Agrotis ypsilon* was more strongly attracted to black light.

The Crambidae also came freely to black and to white lights. About 10 species were noted but only *Nomphila noctuella* was identified.

The Arctiidae were practically all of the species *Halisidota tessellaris* and were taken during July and August. They were equally attracted to black and white light.

The Scarabadaeidae were composed chiefly of small species of the genus *Aphodius.* The traps were not operated until June 10, and unfortunately did not capture species of *Phyllophaga.*

The miscellaneous Coleoptera were chiefly small species such as Nitidulidae. Some elm leaf beetles, lady beetles and cucumber beetles were taken.

The Hymenoptera consisted chiefly of ants although at times parasitic species were taken.

SUMMARY.—When lights of equal wattages were compared, the total catches of insects were slightly greater at white lights. The Hemiptera (chiefly Miridae) respond more freely to white light. The same was true of the Cicadellidae. On the other hand the Trichoptera and the micro Lepidoptera were more strongly attracted to black light. Although the numbers were small, it seems that the Aphididae and the Sphingidae were also attracted more strongly to black light. On the whole, the macro Lepidoptera were about equally attracted to the black and the white light; however, the Tortricidae came somewhat more freely to the black light.

On the basis of foot candles (visibility to man) the black light was far more attractive to insects.

The catches by low watt lamps, from 2 to 15 watts, while not extensive, seem to follow those of high wattage lamps.

In general it does not look as though black light is superior to white light in attracting most insects. However, black light being, almost invisible at short distances, could be used in places where bright white lights are objectionable. If the intensity of the black light were to be increased, the catches would probably be much greater.

The Granulated Type Insecticide for Soil Treatment[1]

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The application of insecticides to crop land by spraying or dusting has not been found too satisfactory. A large portion of the chemical is always lost through drift. To overcome this difficulty granular formulations of insecticides have been developed. With this formulation, the correct amount of chemical may be applied with a minimum loss in material.

There have been many effective methods of control developed for those insects that feed on those parts of plants growing above the ground. However, many of the most destructive insects feed, for at least a part of their life cycle, on those parts of plants growing below the soil surface.

There is now a possible solution to the problem of controlling insects feeding below the soil surface. This has been brought about by the discovery of the new organic insecticides.

Entomologists are now reappraising the control of insects that spend a part of their life cycle in the soil. With the new insecticide chemicals it has been demonstrated that the soil infesting insects can be controlled. Also, that their control has been followed by a marked improvement in the growing crop.

[1] Technical Contribution No. 207 from the South Carolina Experiment Station, Clemson Agricultural College, Clemson, South Carolina.

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The mixing of insecticides with fertilizers has provided an excellent medium, without undue loss, of placing insecticides in the soil. Although mechanically satisfactory, the mixture has been unpopular for many reasons. Among these are the difficulties of administering the fertilizer laws covering grades and registration, labeling and manufacture, and the distribution of the required mixtures. Certain states have prohibited the mixing of insecticides with fertilizers; others will permit the manufacture and sale of such a mixture provided a special tag is attached to each bag. In spite of these handicaps, thousands of tons of fertilizer-insecticide mixtures have been manufactured and sold.

The granular insecticide formulations have been developed to meet this problem. With this type of insecticide available, farmers can purchase an insecticide for use on soil in a bag carrying a registered insecticide label. This can be applied either alone or with fertilizers in any proportion that is considered desirable. The amount of insecticide and method of application will depend on the kind of insects and the dosage recommended.

There are available from the manufacturers inerts sold to extend insecticides, coarse grades that have been marketed for other purposes. Among these is a grade ranging between 30 to 60 mesh size. Experimental formulations indicated that insecticides could be mixed with granular inerts by the same procedures used in the manufacture of dust base concentrates.

Experience has shown that there are many materials that may be used as bases for a granular insecticide. Among these are common clays, highly absorptive clays, bentonite, tobacco by-products and perhaps many others. The selection of the proper base depends on what may be required of the finished product, how it is to be applied, and other related factors. The amount of chemical toxicant absorbed by a granular inert will depend on the physical properties of each inert. To exceed the limit of absorption may produce a non-free flowing product. The lower economic limit of toxicants is about 5 per cent with an upper limit of about 50 per cent. Good working percentages range between 5 and 25 per cent of toxicant to the inert carrier or base.

The distribution pattern of a granular type insecticide will depend on the physical properties of the inert. In this paper the size of the particles termed granular inert are those that pass through a 30-mesh screen, but are held on a 60-mesh screen, termed 30/60 grade. The size of each particle within this range will influence the total number of particles in a pound. It has been calculated that a typical pound sample of 30 to 60 mesh attaclay contains 13,500,000 particles. When equally scattered over an acre, each square foot would average 300 particles, a single square inch 2 plus particles. When calculated against applications of 5, 10, and 15 pounds per acre the particles would be scattered at 10, 20, and 30 per square inch respectively. The individual particles would be about two tenths inch apart when distributed at 30 per square inch.

It may be assumed that as the total pounds of granular insecticides are increased per acre the individual particles will fall closer together. In the manufacture of granular insecticides the total amount of inert base is impregnated with the insecticide. By reasons of the laws of chance, each particle should carry an amount of toxicant in proportion to the size and distribution of that particle within the total mass. When scattered on the soil, as the number of particles are increased per unit of area, the distribution of toxicant would likewise have the same degree of distribution.

In two cases where one pound of technical heptachlor was applied per acre, certain Florida data gave a 96 per cent kill of sand fly larvae following an application of 5 per cent granular heptachlor at 20 pounds per acre; in comparison only a 93 per cent kill was obtained following an application of 10 per cent granular heptachlor at 10 pounds per acre.

The amount of insecticide or toxicant that a granular inert will absorb has an important bearing on the formulation. This factor may vary between 5 and 50 per cent by weight. The objective being to have a finished product that will be free flowing without agitation.

The bulk-weight density of granular inerts may cover a wide range. In tests the most bulky product formulated is a by-product of tobacco manufacture. When formulated at 5 per cent concentration of toxicant it flowed easily and could be handled by all types of equipment. Granular attaclay and similar inerts will absorb up to 25 per cent of certain insecticides. Bentonite has the lowest absorption property of those inerts tested, ranging around 5 per cent. With the exception of granular tobacco by-products all of the formulations tested have a tendency to pack. In the manufacture of granular insecticides the valve packing equipment commonly used for dusts will fail, if the finished products exhibit a property of packing. This property also restricts to a certain extent the type of equipment used for distribution. The addition of a chemical to induce a flow property may correct this difficulty.

A satisfactory base for a granular insecticide should be closely screened for uniformity. An excess of fines will make a dusty formulation that defeats, in part, an important property of granular mixtures. A well formulated mixture will be free flowing and essentially free from dust. Such a mixture when applied will show little tendency to drift, the particles falling directly to the soil. When scattered from altitudes exceeding 100 feet, some formulations may drift slightly with the wind.

The application from an airplane has been very satisfactory over difficult terrain, where the airplane is about the only economical method of applying insecticides. For this purpose a high concentration of toxicant is desirable in order to increase the pay load. Amounts of 10 pounds per acre have been successfully broadcast from the air.

Only experimental flights of the granular formulations will determine the distribution pattern for each type. The heavier bulk density mixtures will give the lesser width of swath covered. In experiments a bentonite formulation flown at 70 feet covered a swath of 27 feet; an attaclay formulation flown at 80 feet covered a swath width of 85 feet; a tobacco by-product formulation flown at 100 feet covered a swath width exceeding 100 feet.

With a Stearman plane, experience has shown the type of pattern that may be expected from a 16 per cent dieldrin formulated into 30 to 60 attaclay. Experimental runs indicated that flying at crop dusting height, this granular insecticide was scattered for the width of the wings or 42 feet. When flown from 80 feet, 85 feet of swath width was obtained.

For perfect coverage of the soil the ship was flown at 85 to 100 feet of altitude following the usual wing width pattern of flight. A flagman stationed at both ends of the run insured a uniform flight pattern. Areas of land flown by this procedure had an es-

sentially uniform distribution of particles on the soil irrespective of the overhead cover of trees, shrubs, grass, or crops.

Other than to wire off the agitator, no changes are necessary on an airplane equipped for dusting, in order to handle granulated insecticides. The flow properties of granulated mixtures are such that they will feed uniformly from an airplane. An experienced pilot can quickly learn the proper feed in accordance with the characteristic of his ship. A full pay load to the capacity of the ship may be carried at each trip.

Tractor driven row crop duster equipment may be used to distribute granular insecticides. The packing characteristic of the material may stop the agitator on some equipment when the hopper is completely filled. Since no agitation is required other than to feed the unit, the agitator may be removed where it gives trouble. Several manufacturers are providing adjustments on their equipment to give less difficulty with this type of material. To obtain a maximum distribution the nozzles must be operated at the highest level from the soil.

For small lots the cyclone grass seeder may be used to cover several acres. For lawns and gardens broadcasting by hand will give satisfactory coverage. With a fertilizer attachment the granulated insecticide may be mixed in the hopper with the fertilizer and applied along with it. The new type fertilizer spreaders work well for broadcasting granular insecticides either alone or mixed with fertilizer or soil lime.

Where it is desirable to place the insecticide in close contact with the insects in the soil, the kinds of insects in the soil, that may be controlled by the method are unlimited. The amount and kind of insecticide applied may be varied within wide limits to suit the particular insect problem.

In the case of late corn heavily infested with corn earworm in the whorl stage, a single application of 5 per cent granular dieldrin or 25 per cent granular toxaphene completely eliminated the infestation within 48 hours. Good reports have been recorded on green June beetles in pastures; corn rootworms in corn and peanuts; sod webworms in pastures and lawns; chinch bugs in pastures and golf courses; ants in lawns; mosquitoes and sand flies in marshes; white-fringed beetles in cultivated and non-cultivated land; and cutworms in gardens and truck crops.

For treating soil, a higher performance per unit of insecticide may be expected where the treatment is worked into the soil. Where the soil is not to be worked up after treatment the amount applied should be doubled per unit of area treated. For pastures and non-cultivated land the following levels have been tried. In pounds technical applied per acre, aldrin 5; dieldrin 2.5, heptachlor 3.75; chlordane 7; toxaphene 25; DDT 25; and parathion 1.

Granulated insecticides should not be confused with dusts in any sense of the word. Under favorable conditions the granular material does not adhere to foliage, but falls through the ground cover on to the soil surface. It should be considered as a means of applying known amounts of insecticides to the soil surface without undue loss of the chemical from drift. The physical character of the product makes it one that may be handled in conventional farm machinery.

SUMMARY.—1. A granular insecticide may be any of the insecticides impregnated on to inerts of 30 to 60 mesh range in particle size.

2. Equipment designed for applying conventional dusts may be used to apply granular insecticides with very little modification.

3. The formulation does not adhere to foliage, but penetrates heavy cover to fall on the surface of the soil.

4. Where the formulation is adaptable it offers promise in the control of many soil infesting insects and in other specialized problems.

Controlling *Anuraphis roseus* and *Aphis pomi*

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The history of the control of *Anuraphis roseus* Baker and *Aphis pomi* Deg. on apple is largely the history of successful insecticide development. Biological control, while very definite and ultimately prevailing, usually arrives too late to be of much practical service to the grower, especially when he is confronted with a serious outbreak of either species. Mechanical controls have never been successfully used. Thus, as better aphicides have been developed, the grower has turned more and more to them for the answer to this problem.

THE ROSY APPLE APHID.—Aphicides directed against the rosy aphid have been used argely as preventives or as insurance against attack. Examples of such usage are: The dinitros or dinitro with oil applied in the dormant period against the eggs; and nicotine sulfate or other materials used in the delayed dormant against the newly hatched stem mothers. The early season oil sprays, while affording some protection, have never been successfully used against severe outbreaks.

For many years entomologists have sought effective aphicides that could be used later in the season against the rosy aphid. This meant one that would penetrate the curled foliage and kill the aphids located there. With the introduction of the phosphorus based materials such aphicides have been found. Data confirming this statement have been in process of collection by different entomologists over the past 5 years, and as examples of such information the reader is referred to table 1.

This table presents summarized data from replicated plots which show the excellent control of the rosy aphid that may now be secured by the phos-

Table 1.—Rosy apple aphid control—Aphids in curled leaves. Wooster, Ohio, 1952.

MATERIALS USED IN 100 GALLONS	PER CENT DEAD
Parathion 15% WP, 1 lb.	100
Malathon 50% Emuls. Conc., 1 pt.	100
Systox[1] 48% Emuls. Conc., 4 ozs.	100
Nicotine sulfate, 1 pt., oil, 1 qt.	93
Check—No spray	2
Materials applied as concentrates	
Parathion 15% WP (×5), 5 lbs.	100
Malathon 50% Emuls. Conc. (×5), 5 pts.	100
Systox 48% Emuls. Conc. (×5), 1¼ pts.	100
Check—No aphicide	4

[1] Systox—Diethoxy thiophosphoric acid ester of 2 (ethylmercapto) ethanol. The Chemagro Corporation.

phorus based materials applied in regular dilutions or in concentrate form. Such spray applications may be made at any time when aphids are present in the curled leaves.

In past years when no material was available to combat the aphids in the curled leaves, entomologists were forced to recommend the preventive or insurance type of spray. This was due to the fact that there were no known means of predicting outbreaks of this species. As the rosy aphid is sporadic in habit and seldom appears in injurious numbers more often than once in four or five years, the question of whether or not to recommend annually the expensive insurance type spray was a very difficult one for entomologists. Now that we have materials for later season use the question becomes, "How late can we apply these sprays and stop commercial damage?" or "Given the advantage of an extra period in which to observe the aphid in the field can an oncoming outbreak be predicted in time for efficient control?"

To the first ot these questions there is now a positive answer. The first aphicidal application should be made in the pink spray and no later. An example of the data on which this answer is founded is given in table 2.

Table 2.—Rosy aphid control—Prevention of injury to fruit. Summarized data from replicated plots. Wooster, Ohio, 1952.

Material and Time of Application	Total Number of Fruits With Aphid Injury[1]
Parathion 15% WP	
0.5 lb. in the pink spray	
1 lb. in the petal-fall spray	18
Parathion 15% WP	
1 lb. in the petal-fall spray	
1.5 lbs. in the first cover spray	256
Check—No aphicide	1030

[1] Total fruits from each treatment averaged 3500.

Data such as shown in table 2 have been collected from replicated plots in considerable amounts. This shows very definitely that delaying the aphicide until the time of the petal-fall spray will not adequately control severe attacks of the rosy aphid whereas its inclusion in the pink spray was very effective.

As to whether or not reliable predictions concerning future abundance of the aphid can be made at the time of the pink spray, some preliminary data suggest that this may be possible. However, further data are necessary before a definite statement to that effect can be made.

Since parathion, if properly timed, is effective against practically all insects and mites, this factor should be considered when it is used against the rosy aphid. For example, excellent control of aphids may be gained by applying parathion in the delayed dormant period. However, if used at that time, no effect is secured against the overwintering eggs of the European red mite whereas if the application is delayed until the pink spray, mites that have hatched will be killed. When parathion is repeated in the petal-fall spray, excellent control of mites for some time is assured. Other insects are also more susceptible to this later application. Thus, by a matter of timing we have literally killed two birds with one stone.

THE APPLE APHID.—Infestations of the apple aphid are very dependent upon the growth condition of the host. Primary infestations, or those that are directly due to aphids hatching from the overwintering eggs, depend upon terminals or water sprouts continuing growth until late in the autumn of the previous year. Such a condition will furnish the necessary food for the aphids that deposit the overwintering eggs. Secondary infestations are started by winged migrants from primary colonies and their establishment, development, and severity are dependent upon the amount of lush growth and the length of time that this persists. Growth conditions are in turn dependent on moisture, temperature, and soil fertility. All these factors plus the tremendous reproductive capacity of the species and the long migration period present the possibilities of a very complex control problem.

From the standpoint of direct chemical control it is fortunate that effective aphicides are available. For many years nicotine sulfate plus different spreaders has answered this purpose and we now have at hand even more effective materials. Summarized results obtained by the use of some of these in replicated field plots are shown in table 3.

Table 3.—Results of field spray tests against the apple aphid. Wooster, Ohio, 1952.

Materials Used in 100 Gallons	Per Cent Dead in 48 Hours	Per Cent Dead in 120 Hours
Parathion 15% WP, 1 lb.	100.0	99.6
Malathon 50% Emuls. Conc., 1 pt.	99.3	100.0
322E (Geigy), 8 ozs.	99.7	100.0
Systox 48% Emuls. Conc., 4 ozs.	100.0	100.0
Nicotine sulfate, 1 pt.+oil, 1 qt.	93.7	99.8
Check—No aphicide	0.0	2.0

While no significant difference can be shown between the performance of several of the materials listed in table 3, experience with Systox in numerous other experiments points it out as a most efficient aphicide.

Due to the interest in concentrate spraying on apples, numerous aphicides have been applied at different concentrations with concentrate sprayers. For example, in mid July 1951, an orchard of fully grown 30 year old trees at Wooster, Ohio was found heavily infested by the apple aphid. Spray records showed that for thorough coverage from 25 to 30 gallons of the regular dilute spray were needed for each tree. A times 10 (×10) concentrate spray, consisting of 10 pounds of parathion 15 per cent wettable powder per 100 gallons of water, was applied using from 2½ to 3 gallons per tree. The aphid mortality throughout the entire orchard following this spray averaged 99.9 per cent. Numerous other trials with concentrate parathion have produced comparable results. Results with Systox as a concentrate have been equally or even more impressive.

Trouble in controlling the apple aphid is not due to the lack of an effective aphicide. It is due to biological and ecological factors such as favorable growth condition of the host, reproductive capacity of the aphid, and migration. These are not likely to be fully understood by the growers. An orchard may develop a severe infestation at a time when terminals are growing and migration is taking place The grower sprays with an effective material and

secures a high mortality. He is highly pleased but growth continues, as does migration, and in about 2 weeks the orchard is found reinfested. The reaction of the grower is well known to all entomologists. He damns the material and usually switches to another or gives up entirely. What he should do is to continue spraying until the time when the infestation does not reappear.

A program of continuous sprays against the apple aphid offers some interesting possibilities. For example, a dosage of 1 pound per 100 gallons of 15 per cent parathion may give 99 per cent mortality while 0.5 pound of the same material will give 97 per cent. When repeat applications are necessary due to biological conditions, practical control may be secured by the use of the lower dosage. On the other hand, if an aphicidal spray was necessary and codling moth and mites also threaten in the orchard, why not step up the dosage of parathion and eliminate all other insecticides from that spray. Numerous possibilities such as this may be considered either in planning a specific program against the apple aphid or a general spray program for all pests.

SUMMARY AND CONCLUSIONS.—(1) Very effective aphicides are now available for use against *Anuraphis roseus* and *Aphis pomi*. (2) The rosy apple aphid may be effectively controlled by sprays applied as late as the pink or latest pre-blossom spray. (3) Predicting outbreaks of the rosy aphid by counts and inspections at the time of the pink spray is a possibility. (4) *Aphis pomi* can be controlled, but due to ecological factors several spray applications of an effective aphicide may be necessary.

A Technique for Mass-Culture of the Six-Spotted Mite

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In 1951 a small colony of *Stethorus vagans* Blackburn, a predatory beetle attacking the two-spotted mite, *Tetranychus bimaculatus* Harvey, was imported from Australia by the Department of Biological Control of the University of California at Albany for the purpose of establishing the species in California.

An insectary program was set up to culture these beetles in sufficient numbers to permit the release of sizeable colonies in the field during the brief period when conditions are optimum in areas representing the different ecological conditions where the two-spotted mite is a pest in California.

Attempts were made in the greenhouse to culture *Tetranychus bimaculatus* on various growing plants, including chard, sugar beets, horse peas, and celery. The celery plant, in exhibiting the greatest tolerance to heavy mite populations, was considered most satisfactory.

However, there were many factors involved that were incompatible with good insectary procedures. Greenhouse temperatures were difficult to control within the desired limits. Flats of plants were bulky, unwieldy units and required extensive greenhouse space for both the backlog of uninfested, growing units and the infested, working units. Other insects attacking the host plants, such as aphids, thrips, and whiteflies would build up in disturbing numbers. The most complicating factor was the appearance of a predatory mite of the genus *Typhlodromus* which built up swiftly under greenhouse conditions to a point where the host-mite infestation was virtually eradicated. This *Typhlodromus* was extremely resistant to TEPP which, because of its brief residual effect, was being used in the control of the greenhouse pests.

An insectary program under the best of conditions is beset with many potential hazards to success. These difficulties are increased many times when a culture of living host-plants is injected into the picture. Consequently, a vigorous search is generally made to find some other type of host material to be used as a substitute. To accomplish this another host insect sometimes must be utilized; even one that the parasite, or predator, does not ordinarily attack in nature.

In line with this principle, a colony of the six-spotted mite, *Tetranychus sexmaculatus* Riley was obtained. This mite can be cultured on ripe market oranges which are much more amenable to insectary manipulation since they can be handled in limited space in small, debris-free units under well controlled conditions. It became much easier to regulate host-predator relationships, since infested oranges could be added to units whenever necessary. The collection of beetle production was also greatly simplified.

However, using this procedure, it was found that a large area of the fruit surface was not utilized by the mites. The desired heavy population was limited to the vicinity of fruit-contact points. This restricted buildup was especially marked when very smooth-skinned oranges were employed. It was also observed that the mites congregated and fed around debris, such as strands of cotton or pieces of string laid across the fruit. This observation led to the idea of applying some short, fibrous, lint-like material to the surface of the orange.

The material first used was made from paper towels which were made into a compact roll about 1.5 inches in diameter and held tightly with rubber bands. The lint was formed by applying the end of this roll to the surface of a slow-turning sanding-disc using a coarse grade of sandpaper. This action reduced the paper to a fibrous lint rather than to an undesirable fine dust. The lint as it was formed was picked up by a tank-type vacuum cleaner.

Small oranges (288 pack) were used because the surface area per box was greater than in the larger sizes. Furthermore small oranges are usually priced much lower than the larger sizes.

The linting unit used in the program at Albany is rather small, accepting only four oranges at a time. The same principle could be applied to larger units with a consequent reduction in the number of operations if the size of the project would make it worth while. The unit consists of a glass jar 18 inches high and 9 inches in diameter. A circular platform made of hardware cloth, 0.5 inch mesh, is suspended about 6 inches from the top. A hole may be cut or depression formed at each fruit position to prevent the oranges from rolling about. The top is made of cloth, either light muslin or organdy, stretched over a frame and laid over the jar.

Prior to use the oranges are held in a cold room or referigerator. Just before they are to be linted they are removed and placed momentarily in a warm room. The slight condensation formed on the fruit is used to catch and hold the lint in place. The oranges are laid in position on their sides on the suspended platform so that there is space between each fruit. A pinch of lint is introduced into the jar and the top put in position. The air hose is placed in contact with the cloth top and a flow of air at a

moderate pressure is directed downard through the cloth along the side of the jar so that the lint within is picked up and circulated upward around the oranges. This operation requires about four seconds and is repeated at about four points around the jar. If the lint has accumulated too heavily on the upper side of the oranges a parting shot of air will carry away the excess.

After this operation the oranges must be handled carefully so as not to disturb the lint. Each fruit should be picked up by grasping it between the thumb and finger at the button and blossom ends.

One difficulty with the use of paper lint was that the material tended to adhere closely to the damp fruit surface and therefore had to be applied very judiciously. If too much was used the purpose of the lint would be defeated by masking the feeding area from the mites.

The Use of Sulfathiazole in Relation to American Foulbrood Disease of Honey Bees[1]

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Many beekeepers have become familiar with the use of sodium sulfathiazole in the treatment of American foulbrood of bees since its use was first reported by Haseman and Childers in 1945. The degree of success resulting experimentally and under general beekeeping conditions in treating this brood disease of bees has been influenced largely by the thoroughness of the methods employed and by conditions existing during the period of the treatment. Our observations have been continued since the previous report[1] in order to determine whether or not satisfactory results could be assured if certain conditions were met during the time the infected colony was under the influence of the medicant.

Our first experiences in treating infected colonies were obtained by attempting to get the colonies to consume or to move all of their natural stores while they were being fed sulfathiazole syrup, on the supposition that if natural stores were moved they would be mixed with the sulfa syrup. This involved reversing the first and second stories of two-story colonies, crushing surfaces of honey stored in the combs, and moving combs from the side walls toward the centers of the brood areas. In some instances, sulfathiazole syrup was shaken into the brood cells, or sulfa-powder was dusted over the brood area and adjacent cells of combs. These methods were sometimes effective when only a small number of brood cells were infected but the disease recurred in other instances when the initial infection was present in a majority of the brood combs. The recurrences apparently were caused by the presence of spores of Bacillus larvae in the natural stores or in brood cells outside of the brood area which were infected before the colony had access to the sulfa-syrup. Under such conditions the disease was eliminated from the active brood area but not from other portions of the combs.

These uncertain results caused us to adopt additional safeguards to assure that *all* stores in the infected colony contained sulfathiazole and that *all* brood cells were thoroughly cleaned by the bees in preparation for brood. Our treatments, then, consisted of extracting[2] all of the original stores of honey from the infected colony and diluting the honey 50 per cent with hot water in which one-half gram of sodium sulfathiazole was dissolved for each gallon of the honey syrup. This medicated syrup was fed back to the colony either by entrance feeders or by the usual ten-pound friction top honey tins, with perforated lids, inverted inside a hive body over the brood chamber. Old capping melter honey or off-grade honey was used as additional feed, when available, and was simply diluted and the sulfathiazole added. Feeding was continued as long as any disease was found in any portion of the hive. During the feeding process, all combs were gradually worked through the center of the brood chamber to force the bees to use all of the old pollen and to get them to clean thoroughly all of the cells for brood. We found that unless this was done, the bees would not clean out all of the AFB scales from brood cells outside of the brood area. In a majority of cases, the bees removed the scales in small pieces without tearing down the cell walls, although in one colony the cells of old, infected combs were torn down to the midrib and then rebuilt.

Sometimes the medicated syrup was shaken into the brood cells at the beginning of the treatment but this took more time, was less exact, resulted in a maximum disturbance of the colony, and invited robbing under certain conditions to a greater extent than when feeding was done by feeders inverted inside of hive bodies placed over the brood chambers.

In hives where the combs were badly infected with AFB, small piles of scale fragments and comb material accumulated on the ground in front of the entrances. These were soon dissipated by the wind or disappeared through other forces. They were not attractive to bees and did not prove a source of infection for other colonies in the apiary.

In order to hasten the production of brood in all combs exposed to the disease in the original colony, two- and three-story colonies were divided and made into separate colonies with laying queens. Two to four pounds of bees from other colonies were added when necessary to make the colonies strong enough to cover a majority of the combs in each hive body. During spring and summer treatments, the surplus medicated syrup was extracted, diluted, the usual amount of sulfathiazole added and then fed back to the colony. This was done to limit the total amount of syrup fed, to make the combs available for brood rearing, and to maintain the stimulus of feeding. In some instances, badly infected brood combs were replaced with frames of foundation or drawn combs.

A chemical analysis of honey extracted from a sulfa-fed colony indicated that the sulfathiazole content had increased from 580 mg. per gallon of syrup to 850 mg. per gallon of honey. This increased amount did not harm the bees or brood when the honey was diluted, additional sulfathiazole added, and then fed back to them.

Frequent inspections were made, and all combs containing any disease were permanently marked with an "A" in the center of the top bar. The divisions were reunited, generally at the beginning of a honey flow or were given supers as needed. Each time the brood chambers were manipulated, the side combs were moved in toward the center of the brood area. The test colonies are kept under observation for one or more seasons after the disappearance of the disease and the tops of the frames marked with

[1] A continuing report from JOUR. ECON. ENT. 40(1): 41–4, 1947.

[2] A small extractor was used for the purpose and was washed thoroughly with hot water to remove all traces of the honey.

paint when the colonies are placed in general production or the combs dispersed to other colonies. As a general practice to test further the freedom of the combs from disease, each colony was divided during the spring following the disappearance of the disease and all combs used as brood combs when the colonies were not under the influence of any medicated syrup.

The length of time it took to free a colony of AFB and the amount of sulfathiazole and syrup that had to be fed under the conditions described varied with the amount of the infection, the distribution of the infection in the combs, the productivity of the queen, the strength of the colony, and the amount of pollen and natural stores available. A colony must have pollen to rear brood, and brood rearing is stimulated greatly by the feeding process if the bees have access to an abundant supply of pollen. The results of 5 years of observations indicated that it is difficult to predict the exact amounts of sulfathiazole or syrup needed to effect a cure as no two colonies are exactly alike. Consequently, the feeding process used may have resulted in feeding, in some instances, greater amounts of sulfa-syrup than might have been needed. Forty-four colonies were fed an average of 3.44 grams of sulfathiazole in five 10-pound tins of syrup over a period of 5 weeks. Ten colonies received from 3.5 to 7.5 grams of sulfathiazole or an average of 5.00 grams, over an average period of 8 weeks. The amount of their infection varied from 10 to 75 per cent, while the infection in all of the colonies treated varied from a few cells to 100 per cent of the brood.

In some colonies a stronger than average sulfa-solution was used without any noticeable benefit. Some periods of feeding were prolonged by the colonies gathering surplus honey, resulting in the bees taking down less of the syrup and in diluting that which they did take down with the incoming nectar. Colonies which were first fed in late summer were carried over until the following spring before a careful inspection could indicate their apparent freedom from the disease. These variations in the conditions in each colony were so great that the average of data secured meant little in estimating the value of the various factors.

In one case, 10 colonies, out of 100 similarly exposed, became lightly infected by robbing diseased combs. The honey was extracted from their combs, and they were fed the sulfa-syrup soon after they first showed signs of being diseased. They cleared up the disease readily within two to four weeks and no additional disease was found in their combs when they were divided and brood reared in all combs the following spring. One of the 10 colonies was found infected in March of the following year with what was later identified as para foulbrood, the first discovery of this disease in California. (It did not improve with the sulfathiazole treatment, although the disease finally did disappear later in the summer after the colony had been requeened twice and strengthened by the addition of four and one-half pounds of bees.)

An Evelyn photoelectric colorimeter was used to determine the amounts of sulfathiazole that occurred in sugar syrup to which one-half gram of sulfathiazole had been added to a gallon of 50/50 syrup and in the honey extracted from the combs after the syrup had been modified by the bees.[3] The results indicated 120 p.p.m. in the syrup and 146 p.p.m. in the modified syrup as extracted from the combs. Royal jelly taken from two-day-old queen cells of a colony fed on sulfa-syrup gave no evidence of sulfathiazole while that from four and four and one-half day old cells indicated 15 and 18 p.p.m., respectively. Worker larvae five days old in the same colony contained 36 p.p.m. of sulfathiazole while queen larvae three and four and one-half days old contained 32 and 46 p.p.m., respectively.

In three colonies found infected with both American and European foulbrood, the sulfathiazole had no effect on the European foulbrood. Twenty colonies that had been in sulfathiazole tests were used later to test disease resistant strains of bees from Molokai, T. H., and from Iowa, so they lost their identity with the sulfa-fed colonies.

CONCLUSIONS.—While it can be demonstrated that colonies infected with American foulbrood can be cured by the feeding of sulfathiazole, it is evident that the procedure must be done thoroughly to be effective. There is no substitute or short cut for thoroughness. The bees did not always clean scales of AFB from cells outside of the active brood area and recurrences of the disease occurred in some cases when original stores of honey were left in the combs during the treatment. It seems obvious that the beekeeper will have to use more than the usual vigilance in inspecting his colonies for AFB during and after the treatment and use a greater amount of hive manipulation than normally required to be assured of satifactory results.

Surplus honey from sulfa-fed colonies may contain sulfathiazole unless the colony consumes all of the syrup fed in the production of brood and beeswax. If diseased colonies are fed during a honey flow, the sulfathiazole will be mixed with the incoming nectar and the resulting product cannot be sold as pure honey. The treatment during a nectar flow also is less effective than during a dearth of nectar, as in early spring or fall.

The question of economy in using the treatment will depend on many conditions as one will have to consider the amount of labor and materials involved, inconveniences and risks of quarantine, contamination of extractors and other warehouse equipment, and holding the colonies out of production for several months to a year or more, as against the cost of destroying the bees and combs and starting over with clean equipment.

Under present California apiary inspection regulations, colonies infected with American foulbrood must be destroyed when found by apiary inspectors, and a similar clause is included in the regulations of a majority of the other states. Most beekeepers in California prefer to destroy what few colonies they find infected with this disease rather than to treat them with sulfathiazole on the grounds that it is cheaper and more satisfactory in the long run.

[3] The chemical determinations were made by H. W. Allinger of the Chemistry Division.

Spittlebug Control with Methoxychlor for Hay Production

J. T. MEDLER, E. H. FISHER and G. W. LYCAN[1]

Methoxychlor is one insecticide among several tested on alfalfa for several years in Wisconsin which is effective for the control of spittlebug nymphs,

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Philaenus leucophthalmus (L). Recent information indicated that methoxychlor presents no serious residue problem on forage. Therefore, in 1952 this insecticide was recommended for spittlebug control on legumes grown for forage in Wisconsin.

In order to demonstrate further the effectiveness of methoxychlor for spittlebug control and obtain data on forage yields affected thereby, spray trials were conducted cooperatively between research and extension workers and interested farmers in 1952.

The program was largely carried on in Sheboygan County because this county is representative of the area where the spittlebug is a serious problem in the eastern part of the state. Early in the spring the County Agent arranged for low-volume spray applications on 11 farms located in south-west, central and north-east parts of the county. Alfalfa fields of about 10 acres each were selected, each field being divided so that half was sprayed and half unsprayed. The large size of the field plots reduced the error caused by variability in plant growth and insect infestation. Methoxychlor emulsion[2] was applied at the rate of 1 lb. technical insecticide in about 10 gallons of water per acre. The height of the alfalfa was about 1 foot; and spittlebugs were just hatching at time of treatment. Insect control was determined by a count of spittle masses on stems selected at random. Yields were determined both by means of half-square rod samples cut and weighed in the field, and the number of hay bales or loads reported by the farmer. The data obtained are presented in table 1.

An examination of data shows that methoxychlor was effective in reducing the infestations of the spittlebug. It should be mentioned that the average infestation in untreated plots of about 50 per cent of the stems was unusually low for eastern Wisconsin. The infestation is commonly 70 to 90 per cent, and greater benefit from insect control would be expected with higher populations. The infestation variation from field to field was caused by different ages of alfalfa stands, the previous year's cutting management and other ecological factors.

Yield data could not be obtained in three fields, but all other treated plots showed increases in production. On the average, the per cent increase was

[2] Acknowledgment is made to the E. I. DuPont de Nemours Co., who provided the methoxychlor (Marlate 2MR) used in the trials.

Table 1.—Spittlebug control and hay yields in Sheboygan Co. alfalfa fields sprayed with methoxychlor emulsion on May 8–9, 1952.

	Treated			Untreated		
Farm	Spittlebug Index[1]	Dry Hay Lbs./A[2]	Bales per Plot	Spittlebug Index[1]	Dry Hay Lbs./A[2]	Bales per Plot
Baack[3]	2	3424		52	2912	
Frauenheim	6	4800		86	4160	
Hinz	0	4352		65	3968	
Johanning[4]	3	3744		36	3616	
Merkel	1	4032		53	3488	
Plautz	8	4960	184	39	3040	162
Depping	0		576	33		503
Conrad	0		400	23		343
Bemis[5]	1			32		
Moth Bros.[5]	1			48		
Gruenwald[5]	6			38		
Ave.	2.5 94.6% decrease	4219 19% increase	387 11% increase	45.9	3531	336

[1] No. infested stems in 100 examined June 3 and 4.
[2] Calculated on basis of ½ square rod samples cut June 19 and 20.
[3] Hay was chopped: the farmer reported 4½ loads on unsprayed, 5½ loads on the sprayed plots.
[4] Hay was chopped: the farmer estimated a 10% increase in loads as result of treatment.
[5] No yield data obtained.

Table 2.—Spittlebug control and hay yields in Washington Co. alfalfa fields sprayed with methoxychlor emulsion on May 20, 1952.

	Treated				Untreated	
	Ground Sprayer		Airplane			
Farm	Spittlebug Index[1]	Yield	Spittlebug Index	Yield	Spittlebug Index	Yield
County Farm	0	Slightly more than 3 loads	2	2.66 loads	38	less than 2 loads
Walsh[2]		350 bales		279 bales		227 bales
Horlamus Bros.	11	2.66 loads	16	2.25 loads	48	1.75 loads

[1] Number of infested stems in 100 examined on June 4.
[2] A driving rain prevented a count of spittlebugs.

greater when determined on June 20, than when plots were harvested at later dates. This would suggest that spittlebug control in conjunction with early harvesting, such as done for grass silage, may be the most economical practice. The more limited increase in yields of hay shown at later harvest dates may be attributed to a re-infestation of the treated plots by leafhoppers, mirids and other injurious insects, or the ability of untreated alfalfa to outgrow to some extent the injury of spittlebugs after the nymphs leave the spittle masses. It is expected that a feed analysis would show important differences in untreated and treated hay at different harvest dates, and this information would be desirable to obtain in future research.

Demonstrations also were conducted on three farms in Washington County in cooperation with the county agent in order to compare the effectiveness of sprays applied by a low-volume ground machine and an airplane; one lb. of technical methoxychlor was applied in about 10 gallons and 5 gallons of spray respectively. Data from these trials are given in table 2. Consistent increase in hay yields were obtained with the methoxychlor treatments. Ground spraying gave better results than airplane spraying probably due in part to the usually less uniform airplane application. The conclusive differences in yields in spite of relatively low spittlebug indexes is explained in part by soil conditions not so favorable to a heavy growth of alfalfa, such as found in Sheboygan County.

Demonstrations in several other counties gave average increases in hay yields of about 10 per cent which is comparable to the results reported.

Notes on the Activity and Control of the Periodical Cicada, 1945 and 1950

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The anticipated heavy emergence of brood X of the periodical cicada, *Magicicada septendecim* (L.), in 1953 throughout many of the eastern States has created wide interest in the activity and present status of control measures for this pest. For this reason, data on the activity and control of brood II in 1945 and brook XXIII in 1950 are summarized below.

Brood II, 1945, Eastern New York. Emergence near Newburgh began a few days prior to May 25. Oviposition began on June 8 and reached its peak June 15–22. In heavily infested areas young fruit trees unprotected by cheese-cloth were completely ruined. Some trees 22 years old had more than 95 per cent of their terminals ruined.

Adults dusted with 3 per cent DDT were not readily affected, and where such a dust was applied directly to the foliage rather than to the cicadas, the mortality was the same as for undusted foliage. A 50 per cent DDT wettable powder, at 4 pounds to 100 gallons, sprayed on a 9-year-old apple block just before the beginning of oviposition had little, if any, effect on the number of adults present or the amount of oviposition. Ferbam, phenothiazine, aluminum sulfate with heavy lime, ryania, and lead arsenate were of no practical value when applied as repellents in sprays.

Brood XXIII, 1950, Southern Indiana. Emergence near Vincennes began May 22 and became heavy by May 25. Oviposition began June 1, reached a peak June 8 to 14, and was completed about June 25. Nymphs hatched between July 24 and September 1. In one apple orchard at least 20,000 adults were present per tree on June 1.

Several growers attempted control, as suggested by Cutright and Parks (1949), with TEPP (40 per cent) at $\frac{1}{4}$ to $\frac{1}{2}$ pint to 100 gallons. In one large orchard the first application killed an estimated 80 per cent of the adults present, and three applications completely cleaned out the infestation. However, most of the applications were spaced a week apart owing for the need of applying the usual orchard cover sprays, and in the areas most heavily infested egg laying was heavy, for adults tended to migrate into the orchards from surrounding woods. Night or early-morning spray applications were the most effective, since adults were quiet at that time.

Sprays applied with sprayers using high air velocities were more effective than those applied with high-pressure hydraulic sprayers. Field and laboratory experiments showed that the adults could be killed with 40-per cent TEPP at $\frac{1}{4}$ pint to 100 gallons or with Metacide (33 per cent) at $\frac{1}{2}$ pint to 100 gallons. To be effective in commercial orchards applications at intervals of 3 days or less would probably be required. Materials that little or no effect on the adults were dialdrin, aldrin, parathion, malathon, and O-(2-chloro-4-nitrophenyl)-O,O-dimethyl thiophosphate (American Cyanamid compound 4124). It was concluded that, for small trees the trunks and scaffolds of which must be protected, the safest and most effective measure is to cover the trees with neeting during the month of June and wrap the trunks with paper. TEPP, if applied frequently enough, will reduce injury to larger trees.

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[1] J. L. Brann, Jr., formerly with the New York (Geneva) Agricultural Experiment Station, cooperated in the studies made in 1945, and S. A. Summerland of this Bureau made many of the activity records for 1950.

An Insecticide-Resistant Strain of the German Cockroach from Corpus Christi, Texas

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In March 1952 it was brought to the attention of the National Pest Control Association that difficulty was being encountered in the control of the German cockroach, *Blatella germanica* (L.), in certain establishments in Corpus Christi, Texas. W. D. Bedingfield, B. & R. Exterminating Company of that city, reported that he had been watching the return of the German cockroach in commercial establishments under his service over a period of a year. He reported finding German cockroaches surviving in spots adequately treated with 2-per cent chlordane oil solution within two weeks of application. From his field observations Mr. Bedingfield concluded that a

[1] National Pest Control Association, Inc., New York, N. Y.
[2] John Powell & Co., Inc., New York, N. Y.
[3] Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture Beltsville, Maryland.

strain of German roaches immune to chlordane residues had developed in his area.

Roaches trapped in the field by Mr. Bedingfield were shipped to the National Pest Control Association and John Powell & Co., Inc., both of whom established laboratory cultures. As soon as the cultures had grown sufficiently in size to permit, tests were made.

Adult male roaches of the first generation reared from field-collected roaches in the laboratories of John Powell & Co., Inc., were tested,[4] using the Tentative C.S.M.A. Test Method for Liquid Roach Sprays, a direct-spray method based on those of Bottimer (1945) and Hazard (1945). A dosage of 0.5 cc. of insecticide was applied using 6 pounds air pressure. The results of these tests, given in table 1, indicated that the Corpus Christi strain possessed a significant degree of resistance to chlordane.

Table 1.—Mortality in 48 hours among adult male German cockroaches from direct-spray test.

Insecticide	Roach Strain	No. Roaches Used	No. Roaches Dead	Per Cent Mortality
2% chlordane	Corpus Christi	16	1	6.2
2% chlordane	Laboratory	16	16	100
OTI (100 mg. pyrethrins/ 100 cc.)	Laboratory	22	21	95.4

Further tests were made on the roaches of the National Pest Control Association culture through the cooperation of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. Laboratory facilities, staff assistance, and roaches from a normal laboratory strain were provided by Frank Babers and R. H. Nelson, Agricultural Research Center, Beltsville, Maryland

Tests were conducted with chlordane, lindane, and DDT on adult males and females reared from Corpus Christi roaches. Direct comparison with the normal laboratory strain was made. The population of the Corpus Christi strain was undoubtedly composed of a mixture of second- and third-generation offspring from field-collected roaches. Specimens of the Corpus Christi strain were identified as *Blattella germanica* (L.) by A. B. Gurney of the Bureau of Entomology and Plant Quarantine.

Tests were made by a measured-drop technique using a micrometer-controlled syringe and blunt hypodermic needle to apply insecticide solution to individual roaches in the amount of approximately .004 ml. per female (average 0.1 g. body weight) and .002 ml. per male (average 0.05 g. body weight). The insecticides were applied in acetone solution. The insecticide dosage was varied through concentration of the solution. Application was made to the ventral surface of the thorax and the coxal membranes. For the application the roaches were anaesthetized under CO_2 gas. Ten roaches of each sex were tested at each dosage level. The roaches were held with food and water for 5 days for mortality counts. The results of these tests are given in Table 2.

From the data of these tests it is apparent that the Corpus Christi strain of roaches possesses an extreme degree of resistance to chlordane (over 100 fold), and significant resistance to lindane (10 to 12 fold) and to DDT (5 to 6 fold). The insecticide resistance of Corpus Christi roaches is such that several pest control operators of the area have been forced to abandon the use of chlorinated-hydrocarbon insecticides and return to the use of sodium fluoride and pyrethrum to effect control.

From no other area of the United States has the National Pest Control Association received reports

Table 2.—Mortality in 5 days among adult male and female German cockroaches from measured-drop applications of insecticide.

Insecticide	Dosage, Mg./Kg.	Per Cent Mortality: Laboratory Strain Male	Laboratory Strain Female	Corpus Christi Strain Male	Corpus Christi Strain Female
Chlordane	2.5	0	10		
	5	0	0		
	10	10	10	10	0
	20	100	40		
	40			0	0
	160			0	0
	640			20	0
	2560			80	20
Lindane	10	70	70		
	20	100	100		
	30	100	100	0	10
	40	100	100		
	60			30	50
	120			90	70
	480			100	100
	1920			100	100
DDT	5	30	20		
	10	10	10	0	0
	20	20	10	0	0
	40	30	30	0	0
	80	80	70	10	0
	160	100	80	60	0
	640	—	—	80	80
	2560	—	—	100	80

of any general difficulty in roach control using chlordane or lindane. Difficulty has been reported for two specific buildings, in Louisiana and Ohio, respectively, but there is no proof that either of these situations has been caused by resistant roaches, although this possibility still exists. No prediction as to the rate at which insecticide resistance may appear in roach populations in other areas of the country can be made at this time.

The major culture of the Corpus Christi roaches has been transferred to the Savannah Laboratories of the Bureau of Entomology and Plant Quarantine.

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Translocation Studies with Two New Phosphate Insecticides

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Publication of the work of Schrader (1947) and subsequent work on systemic insecticides by Ripper

[4] The assistance of Richard Philpitt in the rearing and testing of the roaches is acknowledged.

et al. (1949) and others led to the initiation at this laboratory of simple screening procedures by which the systemic insecticidal and acaricidal activity of new compounds can be compared with known materials.

This paper will describe the systemic acaricidal properties of two new phosphorus compounds, diethyl 2-chlorovinyl phosphate (Compound 1836) and dimethyl 1-carbomethoxy-1-propen-2-yl phosphate (Compound 2046), previously shown to be effective insecticides and acaricides (Corey *et al.*). The physical properties and preliminary determinations of mammalian toxicity are given in table 1.

Limited tests comparing dosages of 0.1 and 1.0 with 0.5 per cent showed definite dosage responses with the same relative rates of toxicity loss at both the higher and lower dosage. The toxicants must, therefore, enter the plants to the full extent in the early minutes after application. Since there is no toxicity increase for a given dosage with longer exposure times, these compounds are thought to move out of the plants in the transpiration stream as David (1951) reports is the case with bis(dimethylamino)fluorophosphine oxide. The rate of loss and length of time that the materials remain in the plants in effective amounts are thought to be a function of their volatility.

Evidence that the materials may be escaping unchanged was obtained in a test with Compound 1836 in which vapors escaping from plants treated by soil application killed mites on adjoining untreated plants when aerial portions of both plants were enclosed under a battery jar but effectively isolated from the treated soil. This indicates also that part of the systemic action may be due to escaping vapors of translocated chemical exerting fumigation effects; action of this type would not be limited to insects feeding on the treated plants.

SUMMARY.—The systemic acaricidal properties of two new organic phosphorus compounds, diethyl 2-chlorovinyl phosphate (Compound 1836) and dimethyl 1-carbomethoxy-1-propen-2-yl phosphate (Compound 2046), are presented.

Data are given which show that Compound 1836 and Compound 2046 as well as Systox, when applied to the lower leaves of broad beans, are quickly translocated to the upper leaves. While Compound 1836 loses its effectiveness after 24 hours and the toxicity of Compound 2046 is significantly reduced, the moderate toxicity of Systox is retained at least for 72 hours. This is thought to be a function of the relative volatility of the compounds.

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Table 1.—Physical properties and preliminary mammalian toxicities of two new phosphorus insecticides.

	COMPOUND 1836	COMPOUND 2046
Name	Diethyl 2-chlorovinyl phosphate	Dimethyl 1-carbomethoxy-1-propen-2-yl phosphate
Structure	$(C_2H_5O)_2P(O)OCH=CHCl$	$(CH_3O)_2P(O)OC(CH_3)=CHCOOCH_3$
Physical State	Colorless liquid	Light yellow-green liquid
Distillation Range	116° C. at 10 mm.	106–107.5° C. at 1 mm.
Evaporation Rate, per cent per hour from glass plates at room temperature	38	3
Stability of diluted emulsible concentrate, measured by bioassay	>7 days	>7 days
Water solubility	Approx. 1%	Miscible
Mammalian toxicity,[1] acute oral LD-50, mg./kg.		
Mice	30.5	8.9
Rats	7.4	4.0
Human red blood cell cholinesterase inhibition,[1] ID-50	1.7×10^{-5}M.	2.3×10^{-6}M.

[1] These data taken from Kodama *et al.*

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